

# **HANDBOOK OF ANALYTICAL METHODS FOR ENVIRONMENTAL SAMPLES**

**VOLUME 2**



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and  
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## THE DETERMINATION OF ORGANIC SOLVENT EXTRACTABLE MATTER

The determination of organic extractable matter is a general test that provides a quantitative measure of sample constituents which are recovered under the selected experimental conditions. A wide variety of organic compounds can be extracted from samples using single or mixed organic solvents, but there is no solvent that selectively extracts a single chemical compound. Other analytical techniques, such as chromatography, are required for compound identification.

Organic solvent extractable substances include non-volatile petroleum hydrocarbons and their derivatives, vegetable oils, animal fats, soaps, greases, and waxes. Thus, this test is commonly called Oil and Grease. When these organics are found in surface waters, they are not only undesirable from aesthetic considerations, they can have a toxic affect on aquatic biota. When such materials are found in sewage and industrial waste streams, they can have a deleterious affect on waste treatment processes.

### Sample Handling and Preservation

#### Water and Sediment

The collection of samples for solvent extractables requires special attention to obtain a sample that is representative of the problem. This is particularly important in the case of composite sampling because of the risk that the organic fraction may adhere to the sampling device. To ensure meaningful data, samplers should discuss potential problems with laboratory staff.

### Selection of Method

The methods presented here are suitable for the quantitative determination of organic substances with common physical characteristics on the basis of solubility in the selected solvent. Two methods are currently used. Method A is a liquid-liquid extraction procedure using separatory funnels for water samples while Method B is a liquid-solid extraction method using Soxhlet extraction units for sediment samples.

### Selection of Solvent

The basic principle in solvent extraction is "like dissolves like". As previously mentioned the technique is not compound selective. A general purpose solvent mixture (diethyl-ether-carbon tetrachloride) had been used for decades but was replaced for safety reasons with Freon 113 (1,1,2-trichloro-1,2,2-trifluoroethane).

Freon 113 is a nonflammable, nonexplosive, chemically pure and stable solvent of very low toxicity. It has an extremely low water solubility and low surface tension to make it a solvent of choice. Due to the relatively high price of Freon 113, methylene chloride was selected as an alternate solvent.

In some specific investigations, when solvent extraction is used as a part of the analytical work, by prior consultation with the staff, the most appropriate and suitable solvent can be selected.

## ORGANIC SOLVENT EXTRACTABLE MATTER

### Liquid-Liquid Extraction Method A

#### SUMMARY

<b>Matrix.</b>	This method is used on drinking water, sewage, industrial waste and leachate samples.
<b>Substance determined.</b>	Substances which are relatively non volatile and soluble in halogenated hydrocarbons.
<b>Interpretation of results.</b>	Results are reported in mg/l. No solvent is known to selectively extract one specific substance. Instead, groups of substances with similar physical characteristics are extracted and quantitatively determined.
<b>Principle of method.</b>	Petroleum products, oils, fats, greases, phenolics, etc. are extracted from water by intimate contact with an organic solvent. The solvent is then removed (with special precautions) and the extractable substances measured gravimetrically.
<b>Time required for analysis.</b>	Results may be obtained within 24 hours.
<b>Range of application.</b>	Variable, from 0 mg/l to %.
<b>Standard deviation.</b>	Not available.
<b>Accuracy.</b>	Not available.
<b>Detection criteria.</b>	Not available.
<b>Interferences and shortcomings.</b>	Losses can occur from the formation of emulsions which result in the loss of extractable matter in the interphase, and from evaporation of the residue during solvent removal.
<b>Minimum volume of sample.</b>	1 liter relatively clear water sample. Less if the suspected material appears to be present in larger amounts. As the entire sample is extracted and the glass container and cap liner are rinsed with solvent, <u>a separate sample must be taken</u> if other tests are required.
<b>Preservation and sample container.</b>	Samples should be stored in a dark, cold room. If the analysis is to be delayed, the sample is preserved by the addition of 5 ml of concentrated hydrochloric acid, and so labelled.

**Safety  
considerations.**

For sample collection, glass containers furnished with aluminum foil lined caps are recommended.

The solvent has a low toxicity and is nonflammable but it is highly volatile. Extraction should be carried out in a well ventilated fume hood avoiding skin contact and breathing of vapours.

## ORGANIC SOLVENT EXTRACTABLE MATTER

### Liquid-Liquid Extraction Method A

#### 1. Introduction

A known volume of sample is extracted with a halogenated hydrocarbon. The organic solvent phase is separated and filtered through anhydrous sodium sulphate into a tared beaker, or other suitable glassware. The solvent is then removed using a rotary evaporator or by gentle heating on a steam bath. The solvent free residue is weighed and the result calculated.

#### 2. Interferences and Shortcomings

Shortcomings of the technique include the oxidation or volatilization of the residue during extraction, formation of emulsions during the separation resulting in the loss of some of the extractable matter in the interface, and loss of matter during solvent evaporation.

#### 3. Apparatus

- 3.1. Drying oven with thermostatic control.
- 3.2. Steam bath of an adequate size to handle "batch" quantities.
- 3.3. Analytical balance sensitive to 0.1 mg.
- 3.4. Fumehood with adequate ventilation.
- 3.5. Desiccator.
- 3.6. Separatory funnels, 1000 and 2000 ml with Teflon stopper and stopcock.
- 3.7. Griffin graduated beaker, 100 ml.
- 3.8. Funnel, short stem, fluted.
- 3.9. Graduated cylinders, 500 ml, 100 ml, 10 ml.
- 3.10. Filter paper, Whatman #42 or equivalent.

#### 4. Reagents

- 4.1. Sulphuric acid ( $\text{H}_2\text{SO}_4$ ), concentrated reagent grade.

4.2. Sodium sulphate, anhydrous ( $\text{Na}_2\text{SO}_4$ ), reagent grade, granular.

4.3. Freon 113 (1,1,2-trichloro-1,2,2-trifluoroethane) b.p.  $48^\circ\text{C}$ .

4.4. Methylene chloride ( $\text{CH}_2\text{Cl}_2$ ) b.p.  $40^\circ\text{C}$ .

4.5. **Sulphuric Acid Solution (50% v/v)**

Prepare a 1:1 (v/v) mixture of concentrated sulphuric acid and distilled water. Slowly add acid to water using cooling if necessary. Eye protective equipment must be worn.

5. **Procedure**

5.1. Place the sample (usually 1 liter) in a separatory funnel of sufficient size to allow agitation after further additions of acid and solvent. Acidify the sample to a  $\text{pH} < 1$  with sulphuric acid solution (50% v/v).

5.2. Rinse sample bottle and cap liner carefully with 15 ml of solvent and add these washings to the separatory funnel.

5.3. Use a total of 100 ml of solvent in 3 equal, successive aliquots. Shake the separatory funnel vigorously for 2 minutes. Periodically release the pressure that is produced in the separatory funnel. Allow the layers to separate.

5.4. Filter the combined organic layers into a tared beaker through a funnel using Whatman #42 filter paper filled with about 15 - 20 g of anhydrous sodium sulphate. Rinse the filter funnel with a small portion of fresh solvent after the last filtration.

5.5. Collect all solvent portions in the tared beaker and place on a steam bath under a fumehood to remove solvent. For minimum oxidation of the extract, in special cases, use a continuous stream of nitrogen gas for solvent removal during the final stages of the evaporation.

5.6. Cool beaker in a desiccator for at least 30 minutes and weigh. A solvent blank must also be carried through this procedure.

6. **Calculation and Reporting**

$$\text{Solvent extractable (mg/l)} = \frac{a - b}{v} \times 1000$$

Where:

a = weight of extract in mg

b = weight of solvent blank in mg

v = sample volume in ml

Results are reported as mg/l with data rounded upward to the nearest mg value. Results  $< 1$  mg/l are reported as NIL.

7. Precision and Accuracy

Not established.

8. Bibliography

8.1. American Public Health Association, American Water Works Association and Water Pollution Control Federation (1975). Standard Methods for the Examination of Water and Wastewater, 14th edition, APHA, Washington, D.C.

8.2. Methods for Chemical Analysis of Water and Wastes. EPA-625/6-74-003a.

## ORGANIC SOLVENT EXTRACTABLE MATTER

### Liquid-Solid Extraction (Soxhlet) Method B

#### SUMMARY

<b>Matrix.</b>	This method is used on soil and sediment, vegetation and air filter samples.
<b>Substance determined.</b>	Substances which are relatively non volatile and which are soluble in halogenated hydrocarbons.
<b>Interpretation of results.</b>	Results are reported in mg extractable residue/kg dried sample.
<b>Principle of method.</b>	Petroleum products, oils, fats, greases, phenolics, etc. are extracted from a dried sample by intimate contact with an organic solvent. The solvent is then removed, and the extractable substances measured gravimetrically.
<b>Time required for analysis.</b>	Results may be obtained within 48 hours.
<b>Range of application.</b>	Variable, from 0 mg/l to %.
<b>Standard deviation.</b>	Not available.
<b>Accuracy.</b>	Not available.
<b>Detection criteria.</b>	Not available.
<b>Interferences and shortcomings.</b>	Variations in the experimental conditions during extraction will affect the data adversely. Drying procedures may lead to losses.
<b>Minimum volume of sample.</b>	100 gm. Although the weight requirement depends on the extent of contamination, 10 - 50 gm sample dry weight is generally analyzed.
<b>Preservation and sample container.</b>	Store samples in cold whenever possible. 16 oz size wide mouth glass jars with aluminum foil lined caps are recommended.



**Safety  
considerations.**

Handle the organic solvents with care despite the low toxicity and non flammable nature of halogenated solvents. Due to their high vapour pressure, use well ventilated fumehoods and avoid contact with the skin and inhalation of vapours.

## ORGANIC SOLVENT EXTRACTABLE MATTER

### Liquid-Solid Extraction (Soxhlet) Method B

#### 1. Introduction

A weighed portion of dried or chemically dehydrated soil and/or solid sample will be extracted with the solvent of choice (Freon®113) using a minimum of 40 extraction cycles. The solute is transferred to a tared flask or beaker and the solvent removed by means of rotary evaporation or on a steam bath. The resulting residue is then weighed.

#### 2. Interferences and Shortcomings

Acceptable results can be obtained only by strict adherence to all details. The homogeneity of the samples, the rate of extraction, solvent removal, and the drying procedure of the residue affect recovery.

#### 3. Apparatus

- 3.1. Soxhlet extractor, appropriate size.
- 3.2. Cellulose thimble/Whatman, appropriate size.
- 3.3. Round bottom flask with ground joint.
- 3.4. Heating mantle.
- 3.5. Vacuum source.
- 3.6. Rotary evaporation system.
- 3.7. Appropriate steam bath.
- 3.8. Fumehood (well ventilated).

#### 4. Reagents

- 4.1.  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  for preparation of "dried" magnesium sulphate for chemical dewatering.
- 4.2. Hydrochloric acid, HCl, concentrated.
- 4.3. 1,1,2-trichloro-1,2,2-trifluoroethane, analytical grade (Brand name Freon®113).
- 4.4. Glass wool (pre-extracted).

#### 5. Procedure

- 5.1. Dry a portion of homogenized solid sample in the most appropriate way.

- 5.2. Weigh an appropriate portion of dried sample into the extraction thimble; 20 g is normally sufficient.
- 5.3. Install Soxhlet extractor and supply 200 ml of solvent to the system.
- 5.4. Extract the sample at a moderate rate (approximately 20 cycles per hour) using a minimum of 40 extraction cycles.
- 5.5. At the end of extraction transfer the solvent into a preweighed flask (rotary evaporation) or tared beaker (steam bath) and remove the solvent.
- 5.6. Cool sample in desiccator for 30 min. and weigh.
- 5.7. A solvent blank is carried through the procedure.

## 6. Calculation and Reporting

$$\text{Solvent extractables: mg/kg dry sample} = \frac{a - b}{w} \times 1000$$

Where:

a = weight of extract in mg

b = weight of solvent blank in mg

w = weight of dried sample in gm

Results are reported as mg/kg dried sample with data rounded upward to the nearest mg value. Results <1 mg/kg are report as NIL.

## 7. Precision and Accuracy

Not established.

## 8. Bibliography

- 8.1. American Public Health Association, American Water Works Association and Water Pollution Control Federation (1975). Standard Methods for the Examination of Water and Wastewater, 14th edition, APHA, Washington, D.C.
- 8.2. Methods for Chemical Analysis of Water and Wastes. EPA-625/6-74-003a.

## THE DETERMINATION OF ORGANOCHLORINE PESTICIDES AND POLYCHLORINATED BIPHENYLS

The organochlorine pesticides (OC's) form a class of compounds which includes widely used insecticides such as DDT. Polychlorinated biphenyls (PCB's) are not used as pesticides but have many industrial uses.

OC's can be classified as halogen derivatives of alicyclic hydrocarbons (BHC, lindane, heptachlor, aldrin, dieldrin, endrin) or aromatic hydrocarbons (DDT, DDD, DDE, endosulfan, HCB, methoxychlor).

The possibility of environmental contamination by organochlorine pesticides and polychlorinated biphenyls is of particular concern in view of their persistence. The stability of many of these products allows them to persist in the environment almost indefinitely creating a special hazard to wildlife due to the capability of many organisms to concentrate these compounds. This means that low levels (ng/l) of OC's and PCB's in water can be biomagnified up the food chain so that fish and fish-eating birds may contain mg/kg levels of these products. PCB's and DDT have been implicated in egg shell thinning in wild birds, endangering survival of the species.

Generally, OC's are resistant to degradation and oxidation, are relatively insoluble in water, have low volatility, and are lipophilic. Similarly, PCB's are inert, have low volatility and are relatively insoluble in water, however, they are also resistant to acids, alkalis and high temperatures. PCB's were used in protective coatings, plasticizers, water proofing compounds, asphalts, inks, casting waxes, adhesives and as dielectrics, hydraulic fluids, grinding fluids and high pressure lubricants.

Generally, the acute toxicity of organochlorine pesticides is low except for aldrin, endrin, toxaphene and endosulfan where the  $LD_{50}$  is in the 12 - 50 mg/kg range.

Maximum permissible levels of most of these compounds have been set or are being set for drinking water, foods and some fish species.

### Sample Handling and Preservation

#### Water

Samples are to be collected in one liter, brown, glass bottles with Teflon or aluminum foil-lined caps, and filled to the mark (approximately 800 ml). These bottles are to be labelled "For PCB and Pesticide Analysis Only". Prior to use the bottles must be solvent washed. This is normally done in the laboratory before sending the bottles to the samplers. The bottles must not be overfilled because extraction is performed directly in the submitted sample bottles. Further transfer or subdivision will cause erroneous results due to adsorption of pesticides onto the container walls. Samples should be refrigerated immediately at 2 - 5°C and remain refrigerated until ready for analysis. The minimum sample size required is 800 ml.

## Fish

Fish tissue should be sampled as soon as possible after the fish is caught. If this proves impossible the fish should be frozen immediately. Whole fish should be individually wrapped in solvent rinsed aluminum foil before freezing. Dissection should be followed by homogenization and refreezing of a suitable aliquot (80 - 100 g) in an aluminum foil cup (rinsed with acetone and allowed to dry prior to use). The minimum sample size is 20 g.

**NOTE:** No contact with plastic bags or other such wrappings can be allowed. Freezing large numbers of fish together must be avoided to prevent cross-contamination.

## Vegetation and Other Biological Material

Vegetation and other biological material must be collected in stringently cleaned glass containers with aluminum foil or Teflon-lined caps. Avoid plastic containers, as plasticizers will leach into the sample and interfere with the analysis. All sample containers must be solvent-washed before use to eliminate contaminants. Normally, this is done in the laboratory prior to sending containers to the samplers.

## Soils and Sediments

Soil and sediment samples are collected in glass jars (16 oz) with screw caps lined with Teflon or aluminum foil. These jars must be solvent washed prior to use. The sample should almost fill the jar to allow thorough mixing before analysis and ensure that a representative aliquot is taken.

The minimum sample size is 100 g although analysis can be performed on a 15 g sample. The larger sample size allows for thorough mixing so that a more representative aliquot can be taken.

## Oils

Samples should be collected in precleaned glass containers with screw caps lined with Teflon or aluminum foil. The sample size is 30 ml although 100 ml is recommended for multi-component oil mixtures.

## **Selection of Method**

Although many techniques have been used for pesticide analysis (TLC, GLC, HPLC, fluorescence, polarography) the extreme sensitivities required for environmental work can be achieved by gas chromatography. This involves the use of selective detectors such as the electron capture detector. Gas chromatography (Method A) is the usual procedure used in pesticide residue analysis.

Of major importance in this type of analysis are the successive extraction, clean-up and separatory steps prior to analysis. The methods used are essentially the same as those generally in use in other pesticide residue laboratories. These are multiresidue techniques, and the various combination of OC's and PCB's requested in different samples, will require the application of different combinations of procedures for each of these steps (clean-up, in particular).

Methods B, C and D are specifically for the determination of PCB's in oil samples. Method B, a rapid screen-gas chromatographic technique, is most useful in emergencies

when a quick answer is required such as in the case of a spill or fire involving oil. The use of fuming sulphuric acid as an oxidizing agent is used as a confirmatory procedure in case all the interferences have not been removed. Method B does not remove the oil and consequently the column and electron capture detector will eventually become contaminated.

Method C, using Florisil clean-up prevents this contamination. This method is essentially the same as Method A except that an extraction procedure is eliminated and GC conditions are different.

Method D, involving a silica-gel clean-up, separates PCB's from aliphatic hydrocarbons which may be present in some samples.

## ORGANOCHLORINE PESTICIDES AND POLYCHLORINATED BIPHENYLS

### Gas Chromatographic Method A

#### SUMMARY

<b>Matrix.</b>	Surface water, domestic water, sewage, industrial waste, leachate, fish, vegetation, soil and sediment samples.	
<b>Substances determined.</b>	<p>Aldrin</p> <p>BHC mixture including <math>\alpha</math>, <math>\beta</math> isomers (see lindane)</p> <p>Chlordane mixture including <math>\alpha</math>, <math>\beta</math> isomers</p> <p>p,p'-DDD</p> <p>p,p'-DDE'</p> <p>o,p'-DDT</p> <p>p,p'-DDT</p> <p>Dieldrin</p>	<p>Endosulphan (isomers I and II and sulphate)</p> <p>Endrin</p> <p>HCB (hexachlorobenzene)</p> <p>Heptachlor</p> <p>Heptachlor expoxide</p> <p>Lindane (<math>\gamma</math> BHC)</p> <p>Methoxychlor</p> <p>PCB (polychlorinated biphenyls)</p>
<b>Interpretation of results.</b>	Results are reported as ng/g (or ng/l). Since most pesticides are associated with agriculture and, to a large degree are bound to particulates in water, high levels may be expected from rural areas, at times of considerable run-off. The most widespread source of PCB's entering the environment is municipal sewage treatment plants but the largest sources are specific industrial discharges or spills.	
<b>Principle of method.</b>	A sample is solvent extracted, dried, concentrated, and cleaned up to remove interferences. The clean extract is examined by electron capture gas chromatography. For water samples, if no PCB's are present, organochlorine pesticides are determined directly on the clean extract by standard calibration and peak height quantitation. PCB's in fish, soil, sediment and water samples are separated from organochlorine pesticides by Florisil column chromatography, enabling the determination of each separately.	
<b>Time required for analysis.</b>	Under optimum conditions 15 - 30 samples per day may be analyzed for PCB's and OC's depending on sample matrix.	
<b>Range of application.</b>	Minimum 1 - 20 ng/l, OC's and PCB's in water. Minimum 1 - 20 ng/g OC's and PCB's in solid samples. Higher concentrations are analyzed after dilution.	
<b>Standard deviation.</b>	Not yet available.	

<b>Accuracy.</b>	Not yet available.
<b>Detection criteria.</b>	Current detection levels do not reflect statistical evaluation of low level replicates but include the minimum measureable instrumental value and an uncertainty factor of 2-5x. Detection levels are more properly called Minimum Measureable Values. 1 - 20 ng/l for water. 1 - 20 ng/g for solid samples.
<b>Interferences and shortcomings.</b>	The simultaneous presence of PCB's and pesticides makes the analysis more difficult, and less accurate, especially when PCB levels are much larger than those of OC's. Normal cleanup procedures will not completely remove interfering sulphur compounds. Electron capture detectors are not element specific, therefore, concrete identification of pesticides often requires confirmation by other means (e.g. chemical derivatization, TLC, GC/MS).
<b>Minimum volume of sample.</b>	<u>Water</u> - 800 ml. The whole sample must be used since adsorption onto the container walls necessitates solvent rinsing to fully recover the pesticides. <u>Solids</u> - e.g. vegetation, sediments, etc.: 20 g dry weight, for fish 80 - 100 g wet weight. Smaller samples can be handled, but with a corresponding decrease in the sensitivity of analysis. The aliquot analyzed is usually 10 g dry weight (sediments) 5 gm wet weight (biota).
<b>Preservation and sample container.</b>	<u>Water.</u> Only 1 liter solvent rinsed glass bottles are acceptable. Cap should be foil or Teflon lined to prevent contamination. Samples should be refrigerated to avoid bacterial degradation and stored in the dark to avoid photodecomposition. Distilled in glass dichloromethane is added as a preservative (80 ml for litre sample). <u>Fish.</u> Samples should be individually wrapped in clean aluminum foil and frozen (-40°C). Freezing of large batches of fish in blocks should be avoided to prevent cross-contamination. <u>Soils, Sediments, and Oils.</u> Samples should be taken in solvent-rinsed 16 oz wide necked glass jars with foil lined caps. Soil and sediment samples should be stored frozen if possible.
<b>Safety considerations.</b>	Since a large number of solvents pose fire and exposure hazards, extreme care must be taken during their transport, storage and use.  The presence of toxic solvent fumes dictates that most extraction and clean-up procedures be conducted within a fume cupboard or under a fume hood.



## ORGANOCHLORINE PESTICIDES AND POLYCHLORINATED BIPHENYLS

### Gas Chromatographic Method A

#### 1. Introduction

The OC's and PCB's routinely analyzed in the laboratory include:

Aldrin	Endosulphan (isomers I and II) and sulphate
BHC mixture including	Endrin
$\alpha$ , $\beta$ isomers (see lindane)	HCB (hexachlorobenzene)
Chlordane mixture including	Heptachlor
$\alpha$ , $\beta$ isomers	Heptachlor epoxide
p,p'-DDD	Lindane ( $\gamma$ BHC)
p,p'-DDE'	Methoxychlor
o,p'-DDT	PCB (polychlorinated biphenyls)
p,p'-DDT	
Dieldrin	

The PCB's include Arochlor 1242, 1248, 1254 and 1260. The OC's and PCB's are extracted from water, fish or sediments with organic solvents. The extracts are then concentrated, cleaned up and separated into various groups using column chromatography and/or liquid/liquid partitions. The cleaned up extracts are concentrated and the OC's and PCB's are determined by gas chromatography with an electron capture detector.

If both PCB's and OC's are present they must be separated by eluting the extract from a Florisil column.

#### 2. Interferences and Shortcomings

Due to the low levels of OC's and PCB's being investigated and the high sensitivity of the electron-capture detector combined with its lack of specificity, the presence of minute amounts of contaminants creates serious problems. Consequently, samples require exhaustive clean-up prior to gas chromatography and extreme care must be taken to avoid contaminating the extracts.

Also, all the glassware used must be thoroughly cleaned by a complex process including acetone washing, detergent washing, rinsing in tap water, followed by distilled water prior to baking overnight in an oven at 300°C. In addition, all glassware is solvent rinsed immediately prior to use. Automatic dish washing has also been shown to be effective after initial acetone rinse.

PCB's interfere with the determination of OC's so that when both are present, a further separation is mandatory.

Some samples (sediments and some waters) contain sulphur and organosulphur compounds which interfere with the gas chromatographic determination and which

are not removed by the normal clean-up procedures. In order to remove sulphur, the extract is shaken with metallic mercury. After the extract has been allowed to settle, the supernatant is reinjected into the gas chromatograph.

Because the electron capture detector used is not element specific, OC's or PCB's must be confirmed by other means. Confirmatory procedures include gas chromatography on several column packings of different polarity, chemical derivatization, and identification according to the elution pattern on the clean-up column. GC/MS is also used regularly for confirmation, where concentrations allow.

### 3. Apparatus

#### 3.1. Extraction Procedure - Water

All glassware must be rinsed with dichloromethane prior to use.

- 3.1.1. Graduated cylinders, 100 ml, 1000 ml.
- 3.1.2. Pipette, transfer, 25 ml.
- 3.1.3. Flask, Erlenmeyer, 250 ml.
- 3.1.4. Cork rings, 60 mm I.D.
- 3.1.5. Filter paper, glass fibre.
- 3.1.6. Rotary, end over end tumbler, 40-60 rpm, fitted to accept 1 liter bottles.
- 3.1.7. Rotary flash evaporator.
- 3.1.8. Filtering funnel.
- 3.1.9. Flask, 250 ml, round bottom, 24/40  $\text{\textcircled{F}}$  joint.
- 3.1.10. Rotary flash evaporator.

#### 3.2. Extraction Procedure - Fish, Biota

All glassware must be rinsed with dichloromethane prior to use.

- 3.2.1. Beaker, 250 ml, Pyrex, graduated.
- 3.2.2. Analytical Balance: top loading, weighing to 0.01 gm.
- 3.2.3. Centrifuge Tubes: 23mm O.D. x 50 cm screw top, teflon lined caps.
- 3.2.4. Flask: 125 ml Erlenmeyer.
- 3.2.5. Filtering funnel.
- 3.2.6. Rotary, end over end tumbler, 30-45 rpm.
- 3.2.7. Volumetric Flask: 100 ml, pennyhead stopper.
- 3.2.8. Pasteur Pipettes: disposable.
- 3.2.9. Glass wool: pre-extracted with dichloromethane.

#### 3.3. Extraction Procedure - Vegetation

All glassware must be rinsed with dichloromethane prior to use.

- 3.3.1. Berzelius beaker, 300 ml.

- 3.3.2. Polytron Ultrasonic Extractor.
- 3.3.3. Kontes vacuum filtration assembly including: Buchner funnel, 150 ml with sintered glass disc and 24/40 joint and vacuum adapter.
- 3.3.4. Separatory funnel, 2 liter, with Teflon stopcock and Teflon stopper
- 3.3.5. Filter paper, glass fibre (Reeve Angel 934 AH) pre-extracted with dichloromethane.
- 3.3.6. Glass wool, pre-extracted with dichloromethane.
- 3.3.7. Rotary flash evaporator.
- 3.3.8. Filtering funnel.
- 3.3.9. Flask, 250 ml, round bottom, 24/40  $\text{\textcircled{F}}$  joint.

### 3.4. Extraction Procedure - Soils and Sediments

- 3.4.1. Berzelius beaker, 300 ml.
- 3.4.2. Sonifier cell disruptor (Heat Systems - Ultrasonics Inc.), 350 Watt.
- 3.4.3. Kontes vacuum filtration assembly including: Buchler funnel, 150 ml with sintered glass disc and 24/40 joint and vacuum adapter.
- 3.4.4. Separatory funnel, 2 liter, with Teflon stopcock and Teflon stopper.
- 3.4.5. Filter paper, glass fibre (Reeve Angel 934 AH).  
pre-extracted with dichloromethane.
- 3.4.6. Glass wool, pre-extracted with dichloromethane.
- 3.4.7. Rotary flash evaporator.
- 3.4.8. Filtering funnel.
- 3.4.9. Flask, 250 ml, round bottom, 24/40  $\text{\textcircled{F}}$  joint.

### 3.5 Clean-up Procedure - All Samples

- 3.5.1. Chromatographic column, Pyrex glass tube, 6 mm I.D. x 280 mm with Teflon stopcock, 12/30 glass joint at top into which fits a 100 ml reservoir.
- 3.5.2. Beaker, Pyrex, 100 ml.
- 3.5.3. Graduated cylinders, 15 ml and 50 ml.
- 3.5.4. Buchler Vortex Evaporator with sample blocks to hold 15 ml and 50 ml conical centrifuge tubes.
- 3.5.5. Centrifuge tubes, 50 ml, Pyrex, graduated, glass stoppered.
- 3.5.6. Centrifuge tubes, 15 ml, Pyrex, graduated, glass stoppered.
- 3.5.7. Pasteur pipettes, disposable, preextraced with dichloromethane.

### 3.6. Moisture Determination - Soils and Sediments

- 3.6.1. Analytical balance weighing to 4 decimal places.
- 3.6.2. Culture tubes, 50 ml, screw caps, Teflon cap liner, Pyrex No. 9825.
- 3.6.3. Centrifuge (2000 rpm +) with head to accept 50 ml culture tubes.
- 3.6.4. Radiometer CDM3 Conductivity Meter, with aspirated continuous flow cell, thermostatted to 25°C.

3.6.5. Hewlett Packard Calculator 9810 A (optional).

### 3.7. Gas Chromatographic Analysis

3.7.1. Gas chromatograph, Hewlett Packard 5713, 5733, 5880 or equivalent with suitable 1 mv F.S.D. recorder and including the following:

3.7.1.1. Column: borosilicate glass, 4 m x 2 mm I.D.

3.7.1.2. Packings: 1.5% + 1.95% mixed phase OV17-QF1 on Gas Chrom Q, 3% Dexil 300 on Chromosorb WHP.

3.7.1.3. Fused silica capillary columns 25-30 m, SE54, DB1701, OV1.

3.7.1.4. Detector: electron capture, radioactive nickel  $^{63}\text{Ni}$ .

3.7.1.5. Syringe (5, 10 and 25  $\mu\text{l}$ ).

3.7.1.6. Hewlett Packard 3354C Laboratory Automation System including full electronic data capture and data reduction from detector signal and automatic sample injector sequencers.

3.7.1.7 Operating conditions.

**NOTE:** Improvements in GLC technology are incorporated frequently. Contact laboratory for most current applications.

Parameter	G.C. Column	Carrier	Flow ml/min	Det $^{\circ}\text{C}$	Inj. $^{\circ}\text{C}$	Col. $^{\circ}\text{C}$
PCB HCB Heptachlor Aldrin ppDDE Mirex	4m x 2 mm ID Glass 3% Dexil 300 on Chromasorb W HP	5% Methane 95% Argon	24	300	250	220
BHC's Chlordanes opDDT ppDDD ppDDD ppDDD	Simultaneous Dual Capillary Column 30m SE54 30m DB1701	Helium (ECD Make-up 5% Methane in Argon)	2 35	300	250	110 - 250 @4 C/min. 8 min hold
Hept. Epox. Thiodan I, II Dieldrin Endrin	Capillary Column 30m OV1	Helium (ECD Make-up 5% Methane in Argon)	3-4 40	300	250	110-250 @4 C/min.

### 4. Reagents

#### 4.1. Extraction Procedure - Water,

4.1.1. Acetone, reagent grade (for rinsing glassware).

4.1.2. Dichloromethane ( $\text{CH}_2\text{Cl}_2$ ), distilled in glass.

- 4.1.3. Sodium sulphate ( $\text{Na}_2\text{SO}_4$ ), granular, anhydrous, pre-extracted with dichloromethane, stored at 130°C.

Note: All solvents used must be pesticide grade or better, and checked, by concentration (300:1) and Gas Chromatography before use.

#### 4.2. Extraction Procedure - Fish

- 4.2.1. Hydrochloric acid: reagent grade, Baker. (Checked by extraction prior to use).
- 4.2.2. Dichloromethane: distilled in glass.
- 4.2.3. Hexane: distilled in glass.
- 4.2.4. Sodium Sulphate: granular, anhydrous, pre-extracted with dichloromethane, stored at 103°C.
- 4.2.5. Sodium Hydrogen Carbonate: reagent, Baker.
- 4.2.6. Solution (25% v/v). Dilute 62.5 ml of methylene chloride to 250 ml with hexane.

#### 4.3. Extraction Procedure - Soil, Sediment, Vegetation.

- 4.3.1. Acetone: distilled in glass.
- 4.3.2. Hexane: distilled in glass.
- 4.3.3. Distilled water: residue free. (Optional)
- 4.3.4. Sodium Sulphate: anhydrous, pre-extracted with methylene chloride, dried and stored at 130°C.
- 4.3.5. Celite 545: residue free.
- 4.3.6. Dichloromethane: distilled in glass.

#### 4.4. Clean-up Procedure Water

- 4.4.1. Sodium sulphate ( $\text{Na}_2\text{SO}_4$ ), granular, anhydrous, pre-extracted with dichloromethane, stored at 130°C.
- 4.4.2. Florisil, Floridin 60-100 PR grade activated at 130°C for at least 1 week before use and stored at 130°C.
- 4.4.3. Dichloromethane: distilled in glass.
- 4.4.4. Hexane: distilled in glass.
- 4.4.5. Dichloromethane Solution (25% v/v) in Hexane.  
Dilute 62.5 ml dichloromethane (distilled in glass) to 250 ml with hexane.
- 4.4.6. Iso-octane: residue free, distilled in glass.

#### 4.5. Clean-up Procedure - Fish, Soils, Sediments and Vegetation.

- 4.5.1. Dichloromethane, distilled in glass.
- 4.5.2. Hexane, Benzene free, distilled in glass (HPLC grade).
- 4.5.3. Dichloromethane solution (25% V/V) in Hexane. Dilute 62.5 ml dichloromethane to 250 ml with hexane.
- 4.5.4. Florisil, Floridin 100-200 mesh, activated at 130°C for at least one week before use and stored at 130°C.
- 4.5.5. Iso-octane: distilled in glass.

- 4.5.6. Mercury (Hg), solvent washed (soil and sediment only).
- 4.5.7. Split check sample: clean fish oil containing known concentrations of PCB (10 µg/ml) and op'DDT (500 ng/ml).
- 4.5.8. 25% diethylether in methylene chloride (v/v).
- 4.6. Moisture Determination - Soils and Sediments**
- 4.6.1. Isopropanol, spectrograde, dry.
- 4.6.2. Sodium chloride, anhydrous, reagent grade powder. This should be finely ground in a ball mill and stored in a dessicator.
- 4.7. Gas Chromatographic Analysis and Standards.**
- 4.7.1. Nitrogen, Helium, 95% Argon + 5% Methane, pre-purified. All Must contain less than 25 ppm oxygen.
- 4.7.2. Air, pre-purified, dry.
- 4.7.3. Benzene, distilled in glass.
- 4.7.4. Iso-octane, distilled in glass.
- 4.7.5. Pesticide standards, reference standards at a purity of 90% of each compound.
- 4.7.6. Hexane, residue free, distilled in glass.
- 4.7.7. **Pesticide Stock Solutions (100 µg/ml)**  
Dissolve 0.010 g of the individual pesticide in 10 ml of benzene and make up to 100 ml in a volumetric flask with benzene.
- 4.7.8. **Standard Pesticide Mixes (ng/ml)**  
Dilute an appropriate aliquot of each stock solution (100 µg/ml) serially with iso-octane to give the following final mixtures and concentrations.

Mix #1

HCB	-	5 ng/ml
Lindane	-	10 ng/ml
(reference peak)		
Heptachlor	-	10 ng/ml
Aldrin	-	10 ng/ml
Octachloro-styrene	-	10 ng/ml
p,p'-DDE	-	25 ng/ml
Mirex	-	50 ng/ml

Mix #2

α-BHC	-	10 ng/ml
γ-BHC	-	10 ng/ml
β-BHC	-	10 ng/ml
α,-chlordane	-	20 ng/ml
γ,-chlordane	-	20 ng/ml
p,p'-DDE		
(reference peak)	-	25 ng/ml
o,p'-DDT	-	20 ng/ml
p,p'-DDD	-	20 ng/ml
p,p'-DDT	-	20 ng/ml

### Mix #3

Aldrin		
(reference peak)	-	10 ng/ml
Heptachlor Epoxide	-	10 ng/ml
Oxychlorane	-	10 ng/ml
Thiodan I	-	10 ng/ml
Dieldrin	-	20 ng/ml
Thiodan II	-	40 ng/ml
Endrin	-	20 ng/ml
Thiodan Sulphate	-	200 ng/ml
Methoxychlor(DMDT)-		200 ng/ml

#### 4.7.9. PCB Stock Solutions (100 µg/ml)

Dissolve 0.010 g of each Aroclor (1016, 1221, 1242, 1248, 1254, and 1260) separately in 100 ml hexane.

#### 4.7.10. PCB Standard and Mixes

Mix appropriate dilutions to give 0.5 µg/ml of each individual Aroclor. in hexane..

Mixtures of Aroclors at a total of 0.5 µg/ml are prepared as dictated by the residues encountered..

## 5. Procedure

All samples require a preliminary extraction and clean up prior to gas chromatographic analysis.

Adsorption chromatography on Florisil is used as a general clean-up procedure and affects certain separation of OC's which may be used to aid in the confirmation of a specific compound.

Large amounts of fat will give low recoveries and alter the elution pattern, therefore, care must be taken to avoid overloading Florisil column.

### 5.1. Extraction Procedure - Water

5.1.1. The sample (received in pre-rinsed, labelled and marked 1 liter or 32 oz glass bottles) is shaken vigorously and some discarded (if necessary) to bring the sample level to that indicated on the bottle (800 ml).

5.1.2. Add 70 ml dichloromethane to the 1 liter bottle (100 ml to 32 oz. bottle). If extraction cannot be performed immediately, shake and store in refrigerator at 2 - 5°C.

**NOTE:** In lab studies have shown this to be an acceptable storage procedure for up to 8 weeks.

5.1.3. Strap bottle on rotary tumbler/extractor and tumble for 15 minutes.

5.1.4. Remove bottle, siphon off lower dichloromethane layer using a 25 ml pipette and suction. Transfer to a 250 ml Erlenmeyer flask.

**NOTE:** Minimal or no water is to be siphoned off. A separate pipette is used for each sample.

5.1.5. Add two individual 40 ml ( 1 Liter bottle) or 50 ml (32 oz. bottle) aliquots of dichloromethane and repeat steps 5.1.3. and 5.1.4.

- 5.1.6. Measure sample volume by emptying each sample into 1000 ml graduated cylinder and noting volume (within 2 ml). (This step can be done later if the analysis so desires).
- 5.1.7. Remove sufficient sodium sulphate from storage oven and allow to cool to room temperature in a beaker. (Approximately 20 ml required per sample).
- 5.1.8. Set up necessary number of filter funnels with filter papers in 250 ml round bottom flasks resting on cork rings.
- 5.1.9. Add cool sodium sulphate to each funnel to a depth of 2-3 cm.
- 5.1.10. Add approximately 10 g cool sodium sulphate to each Erlenmeyer flask and swirl gently to absorb any water present.
- 5.1.11. Filter sample from Erlenmeyer through sodium sulphate in funnel into a 250 ml round bottom flask.
- 5.1.12. Rinse Erlenmeyer with three 5 ml aliquots dichloromethane and pass through sodium sulphate in funnel.
- 5.1.13. Rinse funnel/sodium sulphate with 5 ml dichloromethane into round bottom flask.
- 5.1.14. Discard sodium sulphate in funnel by gently tapping funnel against side of refuse container.
- 5.1.15. Set up round bottom flask on rotary evaporator and concentrate to 1 ml. (Temperature 28°C, 25 mm Hg vacuum).  
NOTE: Do not let samples evaporate to dryness or serious loss of pesticides will occur.
- 5.1.16. Stopper round bottom flask and follow Clean-up Procedure 5.2.
- 5.1.17. Rinse all glassware with cold tap water and reagent surfactant detergent and set appropriate pieces in dishwasher or acetone soaking tanks.

## 5.2. Clean-up Procedure - Water

This procedure eliminates contaminants and the elution sequence separates pesticides into 2 groups. However, PCB's are not separated from DDT's. If the latter are present use Clean-up Procedure 5.6.

Rinse all glassware with hexane prior to use.

- 5.2.1. Assemble chromatographic column with teflon stopcock. (Plug lower end of column with glass wool).
- 5.2.2. Rinse column and reservoir with 5-10 ml hexane.
- 5.2.3. Remove reservoir, close tap, fill column with hexane.
- 5.2.4. Remove Florisil from oven, pour into graduated beaker, the approximate amount required (about 5 ml/column). Allow to cool to room temperature. Add sufficient hexane to cover Florisil.
- 5.2.5. Transfer Florisil slurry into hexane filled column, using either "spoon-shaped" spatula and small powder funnel or Pasteur pipette with tip broken off and small rubber bulb.
- 5.2.6. Pack to height of 16 cm by tapping and draining hexane simultaneously. Do not allow hexane level to drop below Florisil level.



- 5.2.7. Drain hexane until it just reaches top of packing. Rinse down all Florisil from column walls.
- 5.2.8. Rinse column with 30 ml hexane (reservoir required). Close stopcock when hexane just reaches top of packing.
- 5.2.9. Place clean 15 ml centrifuge tube below column to collect eluate.
- 5.2.10. Gently rotate round bottom flask, containing sample extract (5.1.), in palm until approximately 0.2 ml remains, add 0.8 ml hexane. Transfer 1 ml extract to column and allow to drain until sample just reaches top of packing.
- 5.2.11. Measure 15 ml of 25% v/v dichloromethane solution (reagent 4.3.4.) in a graduated cylinder, for elution of column.
- 5.2.12. Rinse round bottom flask (which contained sample) with 1 ml eluant. Pour into column and allow solvent level to just reach top of packing before addition of more solvent. Repeat rinsing twice more (using solvent from the 15 ml eluant).
- 5.2.13. Elute column with remaining eluant (approximately 12 ml). This is fraction A. When eluant just reaches top of packing, close stopcock and change collecting tubes. (Total volume collected - 15 ml).
- 5.2.14. Elute column with 50 ml dichloromethane. This is fraction B.
- 5.2.15. Add approximately 2 ml iso-octane to each fraction and evaporate fractions A and B to 2 ml using the Buchler Vortex Evaporator at settings of 30°C, 22 mm Hg vacuum, and vortex setting of 5 - 6.
- 5.2.16. Make up fractions A and B to 5 ml with iso-octane for gas chromatographic analysis.

<u>FRACTION A</u> contains:	PCB's	o,p'-DDT
	HCB	p,p'-DDE
	Lindane	p,p'-DDD
	Heptachlor	p,p'-DDT
	Aldrin	α, γ Chlordanes
	Octachlorostyrene	α, β BHC
	Mirex	

FRACTION B contains: Oxychlordane  
Heptachlor epoxide  
Endosulfan I, II, sulphate  
Dieldrin  
Endrin  
Toxaphene  
Methoxychlor

**NOTE:** If sample is to be cleaned-up and analysed for PCB only then steps (5.2.11 - 5.2.15) may be deleted and replaced as follows.

- 5.2.17 Measure 20 ml hexane (eluant) in a graduated cylinder.
- 5.2.18 Rinse round bottom (which contains sample) with 1 ml of eluant, transfer to column and allow solvent level to just read top of packing. Repeat rinsing round bottom twice more.
- 5.2.19 Add remaining hexane in graduate to column using reservoir. Allow to collect in centrifuge tube. This is fraction A (PCB).
- 5.2.20. Analyze according to 5.8.

### 5.3. Extraction Procedure - Fish

Most samples arrive for analysis as frozen foil wrapped fillets. Treat large whole fish individually. Wash off adhering mucous and pat dry with paper towel. Measure length to 0.1 cm and weigh to 0.1 g. Cut fish posteriorly along dorsal surface using stainless steel knife. Note sex. Strip epaxial musculature (the muscle above lateral line) from skin and place about 50 g in stainless steel cup and grind for 2 minutes with mechanical homogenizer (Virtis). Using a stainless steel spatula, transfer to aluminum foil cup, cover and designate laboratory number. Store in freezer (-40°C) until analysis.

In the case of small fish, such as minnows, the whole fish is homogenized. Smelt are headed, tailed, gutted and ground.

- 5.3.1. Using stainless steel spatula mix previously ground fish tissue in aluminum foil cup, to ensure homogeneity. Frozen samples should first be thawed for 3-4 hours.
- 5.3.2. Weigh 5 gm ( $\pm 0.1$  gm) of sample into a previously tared centrifuge tube and record sample number and exact weight.  
5 gm is the optimum sample size, smaller samples to a limit of 1 gm can be used when necessary. Maximum sample size is 7 gm.
- 5.3.3. Add 40 ml of concentrated Hydrochloric acid to the fish tissue in the tube and seal using teflon lined cap.
- 5.3.4. Affix tubes to rotary making tumbler ensure caps are securely sealed.
- 5.3.5. Operate tumbler at 30-45 rpm for 1 hour to ensure good dissolution of tissues.
- 5.3.6. Remove tubes from tumbler and add 25 ml 25% dichloromethane in Hexane (v/v). Replace caps securely.
- 5.3.7. Replace tubes on tumbler and extract at 30-45 rpm for 1 hour. Let stand if emulsion develops.
- 5.3.8. Remove upper solvent layer using pasteur pipette and bulb and transfer to erlenmeyer flask.

- 5.3.9. Repeat 5.3.6 - 5.3.8 with a second 25 ml 25% methylene chloride in Hexane (v/v).
- 5.3.10. Dilute acid layer voluminously with water and discard in suitable manner.
- 5.3.11. Add 1 - 2 gm powdered  $\text{NaHCO}_3$  to erlenmeyer containing combined extracts and swirl gently to ensure neutralization.
- 5.3.12. Quantitatively transfer sample extracts from 125 ml erlenmeyer flask to a 100 ml volumetric using a filter funnel, glass fibre filter paper and sodium sulphate. Do not make sample up to the 100 ml mark until ready for cleanup.
- 5.3.13. Stopper flasks and store in appropriate cool, dark area for separation/cleanup.

Samples stored in this manner must be processed through cleanup and separation steps within 24 hours or irreversible precipitation of lipid and protein in the extract may occur and affect final analytical results.
- 5.3.14. Rinse all used glassware under running tap water then with reagent acetone and place in dishwasher or appropriate surfactant detergent bath.

#### 5.4. Clean-up Procedure - Fish

The cleanup procedure used for fish, eliminates unwanted contaminants including lipids and the elution sequence used separates pesticides into three groups. In this procedure PCB's are separated (approximately 95% efficiency) from the DDT's in one step thus eliminating the need for further chromatographic techniques.

- 5.4.1. Make 100 ml volumetric flasks containing sample extract up to the mark. Pipette an aliquot containing equivalent of 1 g fish tissue (usually 20 ml) from volumetric flask. Transfer to graduated test tube and evaporate to oil residue on a Vortex evaporator. Add 1 ml hexanes.

**NOTE:** The natural oil in the sample acts as a keeper and allows this stage of evaporation to be taken to dryness (e.g. no solvent).

**SECOND NOTE:** The Florisil column described will handle up to 80 mg of oil. If the oil residue after evaporation is more than 0.1 ml, a volumetric dilution or alternate aliquot is required to decrease oil to within the 80 mg limit.

- 5.4.2. Assemble chromatographic columns by inserting Teflon plugs and a 0.5 cm glass wool plug in the bottom.
- 5.4.3. Remove Florisil from oven and pour enough to prepare 1 SPLIT CHECK COLUMN into a 100 ml beaker. Return Florisil bottle to oven.
- 5.4.4. Pour Florisil into column and tap gently to form a height of 24 cm.
- 5.4.5. Immediately apply split check sample (4.5.7.) in 1 ml hexanes to top of column.

**NOTE:** Split check comprised of waste fish extracts, fortified to 500 ng/ml op'DDT and 10  $\mu\text{g/ml}$  PCB. (Aroclor 1254:1260 = 4:1).

- 5.4.6. Allow sample to drain just to top of column and add 1.5 ml hexane to top.
- 5.4.7. Continue adding hexane, 1.5 ml at a time until the entire column is wetted.
- 5.4.8. Place reservoir on top of chromatographic column. Add 30 ml hexane to reservoir.
- 5.4.9. Collect a first fraction of 18 ml from the column and then collect 6-10 separate 2 ml fractions. Label tubes with corresponding volumes contained.
- 5.4.10. All fractions are analyzed as in 5.8. This SPLIT CHECK is performed every day to allow for changes in the Florisil activity caused by humidity fluctuation. It must be done to ensure adequate PCB/DDT separation. Compare fractions to op'DDT standard. The split is taken at the first major appearance of op'DDT.
- 5.4.11. Remove Florisil from storage oven and pour enough for 4 columns (approximately 40 ml) into a 100 ml beaker and return Florisil bottle to oven.
- 5.4.12. Pour Florisil from beaker into columns while tamping column to form a 24 cm bed.
- 5.4.13. Place 50 ml centrifuge tube under each column.
- 5.4.14. Immediately apply the 1 ml sample extracts (5.4.1.) to the top of the columns using a Pasteur pipette and rubber bulb.
- 5.4.15. Allow sample to drain onto column until it reaches the top of the Florisil.
- 5.4.16. Rinse sample tube with two 0.5 ml aliquots of hexane and apply these to the column one at a time as above.
- 5.4.17. Add hexane 1.5 ml at a time until entire column is wetted.
- 5.4.18. Place reservoir on top of column and add 30 ml of hexane to reservoir.
- 5.4.19. Collect hexane in 50 ml centrifuge tube to the volume determined in the split check procedure. This is fraction A1.
- 5.4.20. Turn off stopcock and decant excess hexane off top of column into waste receptacle.
- 5.4.21. Remove any remaining hexane using Pasteur pipette.
- 5.4.22. Add 10 ml 25% v/v dichloromethane solution (reagent 4.5.3.). Place 15 ml centrifuge tube under each column.
- 5.4.23. Open stopcock and collect 15 ml. This is fraction A2.
- 5.4.24. Before solvent level reaches top of Florisil bed, remove fraction A2 tube, insert a 15 ml tube, add 10 ml 25% diethyl ether dichloromethane to reservoir. Collect all solvent until column stops dripping. This is fraction A3.
- 5.4.25. Add 2 ml iso-octane to each extract and insert fraction tubes in Buchler Vortex Evaporator as in 5.2.14 and evaporate to 2 ml.

**NOTE:** Do not let samples evaporate to dryness or serious pesticide losses occur.

- 5.4.26. Make up to 5 ml in iso-octane.
- 5.4.27. Analyze samples according to 5.8.

<u>FRACTION A1</u> contains:	PCB HCB Heptachlor Octachlorostyrene	pp'DDE Mirex Aldrin
<u>FRACTION A2</u> contains:	$\alpha$ BHC $\gamma$ BHC (Lindane) $\beta$ BHC $\alpha$ Chlordane $\gamma$ Chlordane	o,p'-DDT p,p'-DDD- p,p'-DDT Toxaphene
<u>FRACTION A3</u> contains:	Oxychlordane Heptachlor Epoxide Thiodan I, II and Sulphate Dieldrin Endrin DMDT	

- 5.4.28. Rinse all used glassware under running warm water and rinse with reagent acetone. Place in dishwasher or appropriate surfactant detergent bath.

#### 5.5. Extraction Procedure - Soils and Sediments (and Vegetation)

All sediment samples should arrive in the laboratory in solvent rinsed glass jars with foil-lined caps. Possible contamination causing unreliable results may arise from other containers. Although this procedure refers only to soils and sediment. Vegetation samples may also be analyzed according to this method by replacing the Sonifier extractor with a Polytron unit.

- 5.5.1. Determine water content of soil or sediment (see Moisture Determination section 5.7.).
- 5.5.2. Weigh appropriate amount ( $\pm 1$  g) of wet sediment into 300 ml Berzelius Beaker to obtain 10 g dry weight (a maximum allowance of 5 g of water will limit amount of very wet sediments) and add 50 ml of acetone.
- 5.5.3. Extract by immersing Sonifier head in sample suspension and operating for 3 minutes. Disperse the heat generated by the homogenizer by cooling the beaker in an ice water bath during the extraction. The increase and decrease in vibration intensity should be gradual to prevent damage to the Sonifier. Rinse Sonifier head with 1-2 ml acetone. Let acetone drip into sample beaker.
- 5.5.4. Allow suspension to settle before filtering.
- 5.5.5. Insert a glass fibre filter and a 2 cm layer of Celite 545 onto the sintered disc of the Buchner funnel and rinse the whole assembly with 20-30 ml acetone. Discard rinsings.
- 5.5.6. Filter sample supernatant through Buchner filter into cylindrical separatory funnel, with vacuum.
- 5.5.7. Add a further 50 ml acetone to remaining sediment and repeat extraction.

- 5.5.8. Filter whole suspension through Buchner funnel, rinsing all sediment into filter using a minimum of acetone (15 ml maximum).
- 5.5.9. Add combined filtrate to a 2 liter separatory funnel containing 1000 ml water. The acetone:water ratio should be a minimum of 1:8.
- 5.5.10. Extract acetone/water 3 times with dichloromethane using one 100 ml portion and two 50 ml portions.
- 5.5.11. Filter the extracts through sodium sulphate into a 250 ml round bottom flask.
- 5.5.12. Evaporate the combined dry extracts to 70-80 ml on a rotary evaporator at 25°C and 25 mm Hg. Adjust vacuum rate of condensation to 1 drop/second.
- 5.5.13. Make up to 100 ml with dichloromethane in a volumetric flask.
- 5.5.14. Measure a 1 g (dry weight) equivalent aliquot into a chentrifuge tube and concentrate to 0.5 ml using the Buckler Vortex Evaporator at 30°C, 22 mm Hg vacuum and a vortex setting of 5-6).

#### 5.6 Clean-up Procedure - Soils and Sediments (and Vegetation)

This clean-up procedure is essentially the same as that described for fish (section 5.4.). The main difference is that the split check sample (5.4.5) does not contain any fish extracts, it is a straight hexanes (HPLC grade) solution fortified at the same levels with PCB and o,p'-DDT. Add the following steps where indicated.

- 5.6.1. Before sample application to column add 0.2 ml, previously washed mercury, to each sample and shake vigorously for 2 minutes. This removes sulphurous compounds.
- 5.6.2. Before submitting samples for gas chromatographic analysis (5.4.27.) again add 0.2 ml mercury and shake for 2 minutes.

#### 5.7. Moisture Determination - Soils and Sediments

This method has been developed as a routine tool for the determination of moisture content in solid samples such as soil. Its application to other materials (fish, vegetation) has not yet been investigated.

- 5.7.1. Tare 50 ml screw cap culture tube (to 4 decimal places).
- 5.7.2. Stir sample until evenly mixed. Weigh 3-5 g soil or sediment into culture tube. Immediately cap tube to prevent moisture loss. Weight should be taken to 5 significant figures. As water content decreases, increase sample weight from 3 to 5 g.
- 5.7.3. Open tubes individually and quickly add 40 ml spectrograde isopropanol. Also add 2 g finely powdered anhydrous sodium chloride to each tube. Also prepare 1 blank containing isopropanol and sodium chloride only.
- 5.7.4. Cap tubes tightly and shake for 2 minutes or until soil is finely suspended.
- 5.7.5. Centrifuge at 2000 rpm for 2 minutes.

- 5.7.6. Read conductivity in  $\mu\text{S}/\text{cm}$  on a Radiometer CDM 3 conductivity meter equipped with an aspiration continuous flow cell, thermostatted at 25°C.
- 5.7.7. Correct conductivity by subtraction of a blank value.
- 5.7.8. Calculate percent water (% W) using the following equation:

$$\% \text{ W} = \frac{6.663601 - \text{Ln}_e \frac{1000}{a}}{2.717683} \times 100$$

b

Where:

a = conductivity in  $\mu\text{S}/\text{cm}$ .

b = sample weight in grams

## 5.8. Gas Chromatographic Analysis

### Gas Chromatography Column Packing Preparation

- 5.8.1. All instruments currently in use are Hewlett Packard 5700 series equipped with  $\text{Ni}^{63}$  electron capture detectors. The detectors are HP's linearized pulse DC and have proven to have a good useful linear range for most compounds under test. All instruments are interfaced and controlled by an HP 3354 lab automation data collection system. Calibration is performed every day for every instrument in use prior to sample analysis.

**NOTE:** It is normal to leave the chromatograph running continuously so as to avoid long stabilization periods and prevent changes in column performance and detector response.

The appropriate calibration mixture is run on the instrument three times or until good replication of values is obtained.

Quantitation is performed based on electronic integration of peak areas. Using the facilities of the HP 3354 data system, methods are prepared and peaks designated with accurate times (to within 0.01 minutes) and amounts (concentrations of instrumental standards). Automatic calibration is performed by the system using the user entered concentration and the area obtained. Response factors (RF) are automatically calculated based on the relation:

$$\text{RF} = \text{Amount/Area}$$

and these peak specific response factors are stored in the analytical methods.

Actual analysis is accomplished by running a sample extract against the prepared methods under exact instrumentally conditions and comparing obtained responses to the method.

A more detailed outline and explanation of these procedures can be found in the Hewlett Packard 3354 data system, Operators Manual (HP #03353-90003).



### 5.8.2. Special PCB Quantitation

Due to the complex nature of PCBs, a single peak quantitation procedure is not performed.

5.8.2.1. A PCB mixture of Aroclor 1254 and 1260 (4:1 at 0.5 µg/ml) is run on the instrument and 21 major peaks are identified and their areas summed.

5.8.2.2. This summed area is then used to determine the response factor which is applied to each peak individually.

Samples compared to this method are accurately quantitated if they contain Aroclors 1254 or 1260 or a mixture of the two.

5.8.2.3. Samples containing other than Aroclors 1254 and 1260 are quantitated by comparing a pure standard of whatever PCB is identified in the sample to the calibration method and correcting for any difference in actual versus reported values, (e.g. Aroclor 1248 standard 1 µg/ml will read 428-484 ng/ml when compared to Aroclor 1254/1260 mixture. This is noted and any sample resembling Aroclor 1248 is corrected by the factor 1000/484).

**NOTE:** This method is a fast and accurate procedure which allows for the widest range of PCB analysis with the minimum of recalibration.

It must be stressed however, that this method is only applicable where the PCB being quantitated is either 1254, 1260, a mixture of 1254 and 1260, or a "full-pattern" PCB of another type (1242, 1248).

Whenever the PCB pattern is significantly altered through degradation of isomer specific loss, or whenever the PCB mixture is extremely complex, this method is only semi-quantitative.

In these cases a sample specific standard or mixture of standard must be prepared and corresponding method prepared for that standard.

### 5.8.3. In-Run Quality Control

5.8.3.1. All analyses are structured on "runs". A run is a distinct set of samples of a given sample type. The run size (i.e. number of samples) varies according to sample type but is typically in the range of 12-25 samples.

Within each run and as part of the complete run size are a series of quality control samples.

- i). Blanks- these are samples which are carried through the entire sample extraction, cleanup and analysis procedure. The only difference between these samples and real samples is; the blank does not include the sample matrix. It is a glassware/reagent blank that monitors the consistency and quality of every procedure and reagent used in a specific analysis technique.
- ii) Spikes - these are, fortified samples of a suitable sample matrix which are again, carried through the entire analytical procedure.



The fortified matrix is chosen based on previous analytical results to contain no detectable (or minimal) residues of the compounds under test. The matrix is then fortified on a daily basis through the addition of 1 ml (via volumetric pipette) of a spiking solution,

The spiking solution is at a concentration of 2 to 20 times that indicated for the instrumental standards. This provides concentrations of ppt (liquid samples) and ppb (solid samples).

The spike solution is usually in acetone or methanol to allow maximum dissolution in the aqueous portion of the majority of samples and to represent as closely as possible a true extraction.

The spikes give a daily within run indication of extraction efficiency and recovery. They also provide necessary confirmation of the efficiency of cleanup/separation techniques (e.g. separation of PCB and DDT group in fish and sediments).

- iii) Duplicates - these are random replicate sub samples of real samples within a run. Whenever possible (as the matrix allows) a double analysis is performed on a single sample to help establish within run deviation.

The only exception to this procedure is waters where the entire sample is required for analysis.

- iv) Standards - these are an extra aliquot of the same standards used for instrumental calibration, which are added to the end of each run on the instrument.

The purpose of these standards is to check on the integrity of residue identity (no significant time drift) and quantitation (no appreciable variation in calibrated response).

5.8.3.2. The following run sizes and QC samples are routinely in use at our labs.

- Fish/Biota - run size - 20
  - 1 Blank
  - 1 Spike
  - 1 Duplicate
  - Standards as required based on analysis undertaken (Instrument specific)
- Water (all types) - run size - 19
  - 1 Blank
  - 2 Spikes
  - Standards (as above)
- Sediment (all types) - run size - 12
  - 1 Blank
  - 1 Spike
  - 1 Duplicate
  - Standards - (as above)

Vegetation - run size - 12  
(all types) - 1 Blank  
                  - 1 Spike  
                  - 1 Duplicate  
                  - Standards - (as above)  
  
Other - run size - as required  
(Air, - 1 Blank  
Formulations)- 1 Duplicate  
                  - Standards - (as above)

**6. Calculation and Reporting**

Results are reported as ng/l or ng/g to 2 significant figures.

**7. Precision and Accuracy**

Data not yet available.

**8. Bibliography**

1. U.S. Environmental Protection Agency. 1976. Analysis of Pesticide Residues in Human and Environmental Samples. Environmental Toxicology Div., Health Effects Laboratory, Research Triangle Park, N.C.
2. U.S. Department of Health Education and Welfare. 1965. Guide to the Analysis of Pesticide Residues - Volumes 1 and 2. Public Health Service, Bureau of State Services (Environmental Health), Office of Pesticides, Washington, D.C.

## DETERMINATION OF POLYCHLORINATED BIPHENYLS IN OILS

Rapid Scan Gas Chromatographic Method B  
Routine Scan Gas Chromatographic Method C  
Silica Gel - Gas Chromatographic Method D

### SUMMARY

<b>Matrix.</b>	This method is used to determine PCB's in oils: road oils, transformer fluids, diffusion pump oils, hydraulic fluids.
<b>Substance determined.</b>	Polychlorinated biphenyls (PCB's).
<b>Interpretation of results.</b>	Results are reported as $\mu\text{g/g}$ as PCB. This method is useful in emergency spills and fires involving oil.
<b>Principle of method.</b>	PCB's are determined on cleaned samples by electron capture gas chromatography. A Florisil column removes any oxidized material as well as any polar compounds in the waste material. Sulphuric acid oxidation is employed when further clean-up is required.
<b>Time required for analysis.</b>	Total length of time required for analysis is 1 day.
<b>Range of application.</b>	Minimum 5 $\mu\text{g/g}$ PCB's. Higher concentrations are analyzed after appropriate dilution.
<b>Standard deviation.</b>	Not yet available.
<b>Accuracy.</b>	Not yet available.
<b>Detection criteria.</b>	5 $\mu\text{g/g}$ .
<b>Interferences and shortcomings.</b>	The rapid scan Method B does not remove oil and will eventually contaminate the gas chromatographic column and electron capture detector. The presence of sulphur compounds in oil can interfere with identification of PCB's by gas chromatography.
<b>Minimum volume of sample.</b>	30 ml.
<b>Preservation and sample container.</b>	Pre-cleaned glass containers with screw caps lined with Teflon or aluminum foil are recommended. No preservative is required.

**Safety**  
considerations.

Normal laboratory safety precautions. Care should be exercised when handling solvents.

## DETERMINATION OF POLYCHLORINATED BIPHENYLS IN OILS

### Rapid Scan Gas Chromatographic Method B

#### 1. Introduction

This rapid method for PCB analysis in oil is used in cases of emergency. A quick clean-up is effected prior to analysis by electron capture gas chromatography.

#### 2. Interferences and Shortcomings

This method will cause eventual contamination of the gas chromatographic column and electron capture detector. The presence of sulphur compounds in oil can interfere with the identification of PCB's by gas chromatography. Sulphuric acid oxidation is required when all interferences have not been removed.

#### 3. Apparatus

- 3.1. Pasteur pipettes, disposable.
- 3.2. Graduated cylinders, 25 ml.
- 3.3. Beaker, 100 ml, Pyrex.
- 3.4. Centrifuge tube, 15 ml, Pyrex, graduated.
- 3.5. Kontes blowdown apparatus or equivalent.
- 3.6. Water bath (for sulphuric acid treatment only).
- 3.7. Volumetric flasks, 100 ml.
- 3.8. Glass wool, solvent rinsed.
- 3.9. Gas chromatograph, Tracor 222 or equivalent with the following:
  - 3.9.1. Column: Pyrex glass, 6 ft x 2 mm I.D.
  - 3.9.2. Packing: 3% Dexsil on Chromosorb W.
  - 3.9.3. Detector: electron capture - Nickel <sup>63</sup>.
  - 3.9.4. Syringe: Hamilton, 10 µl.

**NOTE:** All glassware must be rinsed thoroughly with hexane and checked by gas chromatography before use.

#### 4. Reagents

- 4.1. Hexane ( $\text{CH}_3(\text{CH}_2)_4\text{CH}_3$ ), Caledon, residue-free, distilled in glass.

- 4.2. Florisil, 60/100 mesh, activated at 130°C.
- 4.3. Sulphuric acid ( $\text{H}_2\text{SO}_4$ ), concentrated, reagent grade (for sulphuric acid treatment).
- 4.4. Sulphuric acid ( $\text{H}_2\text{SO}_4$ ), fuming (15%) (for sulphuric acid treatment).
- 4.5. Sodium sulphate ( $\text{Na}_2\text{SO}_4$ ), granular, anhydrous.
- 4.6. Benzene ( $\text{C}_6\text{H}_6$ ), Caledon, residue-free, distilled in glass.
- 4.7. Polychlorobiphenyl (PCB): a range of PCB products (Aroclors 1242, 1248, 1254, 1260, etc.) should be obtained to enable the sample to be matched with and measured against a standard of similar composition.
- 4.8. **PCB Stock Solution (100  $\mu\text{g/ml}$ )**  
Dissolve 10 mg PCB in a few ml benzene and make up to 100 ml in hexane.
- 4.9. **PCB Working Solution (1  $\mu\text{g/ml}$ )**  
Dilute 1 ml stock solution to 100 ml with hexane.

## 5. Procedure

### 5.1. Chromatographic Cleanup

- 5.1.1. Weigh 20 mg oil sample and dissolve in 1 ml hexane.
- 5.1.2. Plug end of disposable Pasteur pipette with glass wool. Add Florisil/hexane slurry until packing in pipette is 4 cm high.
- 5.1.3. Elute column with 5 ml hexane and discard.
- 5.1.4. Place 15 ml centrifuge tube below Pasteur pipette to collect eluate.
- 5.1.5. Transfer sample to top of column using a disposable pipette.
- 5.1.6. Elute with 10 mls hexane.
- 5.1.7. Evaporate eluate to 2 ml by placing centrifuge tube in Kontes blowdown apparatus prior to gas chromatographic analysis.

### 5.2. Fuming Sulphuric Acid Cleanup

This method is used as a confirmatory procedure used in conjunction with 5.1. in case all interferences have not been removed. This method separates PCB's from oils.

- 5.2.1. Prepare a 1:1 mixture of fuming sulphuric acid (15%) and concentrated sulphuric acid.
- 5.2.2. Evaporate a suitable aliquot of standard or sample (1 ml) to dryness in a 15 ml tube.

- 5.2.3. Add 0.5 ml acid solution and stopper. Place in a heated water bath at 75°C for 15 minutes.
- 5.2.4. Cool and carefully add 10 ml distilled water.
- 5.2.5. Add 1 ml hexane, shake for 2 minutes and allow to settle.
- 5.2.6. Remove hexane layer.
- 5.2.7. Dry hexane over sodium sulphate.
- 5.2.8. Inject suitable aliquot into gas chromatograph (activated Florisil cleanup may be required).
- 5.2.9. Analyze according to 5.3.

### 5.3. Gas Chromatographic Analysis

- 5.3.1. Operating conditions:  
 Injector: 220°C  
 Detector: 300°C  
 Column oven: 185°C  
 Gas flow: 40 ml/min pre-purified nitrogen.  
 NOTE: For gas chromatographic set-up, see 5.8, Method A.
- 5.3.2. Inject 5 µl sample.
- 5.3.3. If resulting chromatogram is off scale, dilute sample with hexane until a 5 µl aliquot gives a chromatogram with a 50% full scale deflection.
- 5.3.4. Inspect chromatogram and determine which PCB if any is present.
- 5.3.5. Inject 5 µl of corresponding PCB standard and compare to sample.
- 5.3.6. If sample and standard correspond, calibrate instrument by injecting a 3 µl and a 2 µl aliquot of standard.

## 6. Calculation and Reporting

- 6.1. Plot a calibration curve using total peak height of the major compounds equivalent to 2, 3 and 5 ng of PCB (injection of 2, 3 and 5 µl).

Measure corresponding peak heights in sample and read the PCB value from the graph.

- 6.2. Calculate PCB concentration using the following formula. Results are expressed as ppm PCB.

$$\text{ppm PCB} = \frac{W_1 \times V_2}{V_1 \times W_2}$$

Where:  $W_1$  = ng PCB from graph

V 1 = volume of extract injected (  $\mu$ l)  
W 2 = weight of sample (gm)  
V 2 = total volume of diluted sample (ml)

## 7. Precision and Accuracy

The overall recovery of PCB from oil using these procedures has been in the 90-100% range for samples containing up to 1% PCB. If higher concentrations are suspect, dilute the 20 mg sample with iso-octane to a suitable volume and from this, apply the 1 ml aliquot to the silica gel column.

## 8. Bibliography

1. U.S. Environmental Protection Agency. 1976. Analysis of Pesticide Residues in Human and Environmental Samples. Environmental Toxicology Div., Health Effects Laboratory, Research Triangle Park, N.C.
2. U.S. Department of Health Education and Welfare. 1965. Guide to the Analysis of Pesticide Residues - Volumes 1 and 2. Public Health Service, Bureau of State Services (Environmental Health), Office of Pesticides, Washington, D.C.



## DETERMINATION OF POLYCHLORINATED BIPHENYLS IN OILS

### Routine Scan Gas Chromatographic Method C

#### 1. Introduction

This method is the normal procedure used for PCB analysis in oils, when the time factor is not critical. Samples are cleaned up by Florisil column chromatography. Elution with hexane removes oxidized material and interferences. The eluate is analyzed by electron capture gas chromatography.

#### 2. Interferences and Shortcomings

This method does not remove oil and will cause eventual contamination of the gas chromatographic column and electron capture detector. The presence of sulphur compounds in oil can interfere with the identification of PCB's by gas chromatography. Sulphuric acid oxidation is also employed when all interferences have not been removed (Method B: 5.2.). Eluting silica gel with selective solvent separates PCB's from the oils (Method D: 5.1.).

#### 3. Apparatus

- 3.1. Pasteur pipettes, disposable.
- 3.2. Graduated cylinders, 25 ml.
- 3.3. Beaker, 100 ml, Pyrex.
- 3.4. Centrifuge tube, 50 ml, Pyrex, graduated.
- 3.5. Kontes blowdown apparatus or equivalent.
- 3.6. Water bath (for sulphuric acid treatment only).
- 3.7. Volumetric flasks, 100 ml.
- 3.8. Glass wool, solvent rinsed.
- 3.9. Gas chromatograph, Tracor 222 or equivalent with the following:
  - 3.9.1. Column: Pyrex glass, 6 ft x 2 mm I.D.
  - 3.9.2. Packing: 3% Dexsil on Chromosorb W.
  - 3.9.3. Detector: electron capture - Nickel<sup>63</sup>.
  - 3.9.4. Syringe: Hamilton, 10  $\mu$ l.

**NOTE:** All glassware must be rinsed thoroughly with hexane and checked by gas chromatography before use.

#### 4. Reagents

- 4.1. Hexane ( $\text{CH}_3(\text{CH}_2)_4\text{CH}_3$ ), Caledon, residue-free, distilled in glass.
- 4.2. Florisil, 60/100 mesh, activated at  $130^\circ\text{C}$ .
- 4.3. Sulphuric acid ( $\text{H}_2\text{SO}_4$ ), concentrated, reagent grade (for sulphuric acid treatment).
- 4.4. Sulphuric acid ( $\text{H}_2\text{SO}_4$ ), fuming (15%) (for sulphuric acid treatment).
- 4.5. Sodium sulphate ( $\text{Na}_2\text{SO}_4$ ), granular, anhydrous.
- 4.6. Benzene ( $\text{C}_6\text{H}_6$ ), Caledon, residue-free, distilled in glass.
- 4.7. Polychlorobiphenyl (PCB): a range of PCB products (Aroclors 1242, 1248, 1254, 1260, etc.) should be obtained to enable the sample to be matched with and measured against a standard of similar composition.
- 4.8. **PCB Stock Solution (100  $\mu\text{g}/\text{ml}$ )**  
Dissolve 10 mg PCB in a few ml benzene and make up to 100 ml in hexane.
- 4.9. **PCB Working Solution (1  $\mu\text{g}/\text{ml}$ )**  
Dilute 1 ml stock solution to 100 ml with hexane.

#### 5. Procedure

##### 5.1. Chromatographic Cleanup

- 5.1.1. Weigh 2.0 g of oil sample and dissolve in methylene chloride.
- 5.1.2. Transfer solution to 100 ml volumetric flask and adjust to mark.
- 5.1.3. Take a 1.0 ml aliquot, add 1 drop of mercury, shake and carefully evaporate to remove methylene chloride.
- 5.1.4. Add 1 ml hexane, shake and allow to stand.
- 5.1.5. To chromatographic column add a plug of glass wool and rinse with hexane.
- 5.1.6. Remove Florisil from oven, pour into baker the approximate amount required (about 5 ml/column). Allow to cool for 2 minutes and add sufficient hexane to cover Florisil.
- 5.1.7. Transfer Florisil slurry into hexane filled column, using a Pasteur pipette with tip broken off.
- 5.1.8. Pack to height of 16 cm by tapping and draining hexane simultaneously. Drain hexane until it just reaches top of packing.

**NOTE:** DO NOT ALLOW HEXANE LEVEL TO DROP BELOW FLORISIL LEVEL.

- 5.1.9. Rinse column with 30 ml of hexane (reservoir required). Close stopcock when hexane just reaches top of packing.
- 5.1.10. Place clean 50 ml centrifuge tube below column to collect eluate.
- 5.1.11. Transfer the 1 ml of sample extract (5.1.4.) to column and allow to drain until sample just reaches top of packing.
- 5.1.12. Measure 20 ml of 1% v/v benzene in hexane in a graduated cylinder, for elution of column.
- 5.1.13. Rinse centrifuge tube (which contained sample) with 1 ml eluant.
- 5.1.14. Pour into column and allow solvent level to just reach top of packing before addition of more solvent.
- 5.1.15. Repeat rinse twice more using eluant.
- 5.1.16. Elute column with remaining eluant.
- 5.1.17. Evaporate the eluate (approximately 17 ml to 1 ml), using a stream of dry oil-free air.
- 5.1.18. Add 0.1 ml of mercury and shake to remove any sulphur present.
- 5.1.19. Analyze according to 5.2.

**NOTE:** If Florisil clean-up does not remove all interferences, proceed with sulphuric acid clean-up procedure 5.2., Method B.

## 5.2. Gas Chromatographic Analysis

- 5.2.1. Operating conditions:  
Injector: 220°C  
Detector: 300°C  
Column oven: 185°C  
Gas flow: 40 ml/min pre-purified nitrogen.  
**NOTE:** For gas chromatographic set-up, see 5.8, Method A.
- 5.2.2. Inject 5 µl sample.
- 5.2.3. If resulting chromatogram is off scale, dilute sample with hexane until a 5 µl aliquot gives a chromatogram with a 50% full scale deflection.
- 5.2.4. Inspect chromatogram and determine which PCB if any is present.
- 5.2.5. Inject 5 µl of corresponding PCB standard and compare to sample.
- 5.2.6. If sample and standard correspond, calibrate instrument by injecting a 3 µl and a 2 µl aliquot of standard.

## 6. Calculation and Reporting

- 6.1. Plot a calibration curve using total peak height of the major compounds equivalent to 2, 3 and 5 ng of PCB (injections of 2, 3 and 5  $\mu$ l).

Measure corresponding peak heights in sample and read the PCB value from the graph.

- 6.2. Calculate PCB concentration using the following formula. Results are expressed as ppm PCB.

$$\text{ppm PCB} = \frac{W_1 \times V_2}{V_1 \times W_2}$$

Where:  $W_1$  = ng PCB from graph  
 $V_1$  = volume of extract injected ( $\mu$ l)  
 $W_2$  = weight of sample (gm)  
 $V_2$  = total volume of diluted sample (ml)

## 7. Precision and Accuracy

The overall recovery of PCB from oil using these procedures has been in the 90-100% range for samples containing up to 1% PCB. If higher concentrations are suspect, dilute the 20 mg sample with iso-octane to a suitable volume and from this, apply the 1 ml aliquot to the silica gel column.  
Data not yet available.

## 8. Bibliography

1. U.S. Environmental Protection Agency. 1976. Analysis of Pesticide Residues in Human and Environmental Samples. Environmental Toxicology Div., Health Effects Laboratory, Research Triangle Park, N.C.
2. U.S. Department of Health Education and Welfare. 1965. Guide to the Analysis of Pesticide Residues - Volumes 1 and 2. Public Health Service, Bureau of State Services (Environmental Health), Office of Pesticides, Washington, D.C.

## DETERMINATION OF POLYCHLORINATED BIPHENYLS IN OILS

### Silica Gel Gas Chromatographic Method D

#### 1. Introduction

PCB's are separated from oils using column chromatography by eluting with selective solvents. The eluate is then analyzed by gas chromatography using an electron capture detector.

This method separates the PCB's from the oil and prevents contamination of the gas chromatographic column and electron capture detector.

#### 2. Interferences and Shortcomings

As PCB's may be present in percentage concentrations, care must be taken to avoid cross contamination of samples, especially through improper glassware cleaning.

The presence of sulphur compounds in oil can interfere with the identification of PCB's by gas chromatography. Methods for the removal of sulphur are described.

The silica gel used in the chromatographic separation of PCB's must be maintained in a fully activated state, otherwise poor fractionation will occur and hydrocarbons will be recovered in the PCB fraction and vice versa.

#### 3. Apparatus

3.1. Centrifuge Tube, Pyrex 15 ml, graduated.

3.2. Beaker, Pyrex, 100 ml.

3.3. Pasteur Pipettes, disposable.

3.4. Graduated Cylinders, 15 ml.

3.5. Kontes Blowdown Apparatus, or equivalent.

**NOTE:** All glassware must be rinsed thoroughly with hexane and checked by gas chromatography before use.

3.6. Gas chromatograph, Tracor 222 or equivalent, including the following:

3.6.1. Column: Pyrex glass, 6 ft x 2 mm I.D.

- 3.6.2. Packing: 3% Dexsil on chromosorb W.
- 3.6.3. Detector: electron capture - Nickel<sup>63</sup>.
- 3.6.4. Syringe: Hamilton, 10  $\mu$ l.

3.7. Chromatographic columns, Pyrex glass tube, 6 mm I.D. x 140 mm with Teflon stopcock at bottom 12/30 glass joint at top in which fits a 100 ml reservoir.

#### 4. Reagents

- 4.1. Iso-octane ( $(\text{CH}_3)_3\text{C} \cdot \text{CH}_2 \cdot \text{CH}(\text{CH}_3)_2$ ), Caledon, residue-free, distilled in glass.
- 4.2. Benzene ( $\text{C}_6\text{H}_6$ ), residue-free, distilled in glass.
- 4.3. Hexane ( $\text{CH}_3 \cdot (\text{CH}_2)_4 \cdot \text{CH}_3$ ), Caledon, residue-free, distilled in glass, HPLC grade.
- 4.4. Silica Gel 100-200 mesh (Merck). Stored and activated at 130°C.
- 4.5. Polychlorinated biphenyl (PCB). A range of PCB products should be obtained to enable the sample to be matched with, and measured against a standard of similar composition.
- 4.6. **PCB Stock Solution (100  $\mu$ g/ml)**  
Dissolve 10 mg PCB in a few ml of benzene. Make up to 100 ml with hexane.
- 4.7. **PCB Working Solution (1  $\mu$ g/ml)**  
Dilute 1 ml PCB stock solution to 100 ml with hexane.
- 4.8. PCB spike solution add 1 ml of PCB stock solution (100  $\mu$ g/ml) to 2.0 g of 10W/30 engine oil make up to 100 ml with hexane.

#### 5. Procedure

##### 5.1. Clean-up Procedure

- 5.1.1. A PCB spike determination (1 ml of PCB spike solution) should be prepared prior to sample analyses to evaluate the specific solvent split required for PCB separation.
- 5.1.2. Rinse column and reservoir with iso-octane.
- 5.1.3. Plug lower end of column with glass wool and rinse with iso-octane.
- 5.1.4. Remove silica gel from oven, pour required amount into a beaker, allow to cool for 3-5 minutes. Add sufficient iso-octane to cover.
- 5.1.5. Transfer silica gel slurry into column using Pasteur pipette with tip broken off.

- 5.1.6. Pack to a height of 6" by tapping and draining iso-octane simultaneously. (Do not allow solvent layer to drop below level of silica).
- 5.1.7. Drain out excess solvent until it just reaches the top of the packing. Close tap.
- 5.1.8. Place 15 ml centrifuge tube below column to collect eluate.
- 5.1.9. Weigh 20 mg of sample, dissolve in 1 ml iso-octane.
- 5.1.10. Transfer the pre-weighed sample in 1 ml iso-octane to top of column using a disposable pipette.
- 5.1.11. Measure 15 mls of iso-octane in graduated cylinder for elution of the column.
- 5.1.12. Rinse beaker with 1 ml of eluate and transfer to the column.
- 5.1.13. Open tap and elute column with remainder of iso-octane.
- 5.1.14. When solvent level reaches top of packing, close stopcock, remove centrifuge tube (A) and replace with another (B).
- 5.1.15. Add 15 mls benzene to column, open stopcock and collect eluate.
- 5.1.16. Evaporate eluates A and B to dryness, add 1 ml hexane to fraction B prior to gas chromatographic analysis.
- 5.1.17. Add 0.1 ml mercury, shake to remove any sulphur present. As described above, PCB's will be eluted from a silica gel column by benzene (tube B). The iso-octane will elute any aliphatic hydrocarbons present (tube A).
- 5.1.18. Analyze according to 5.2.

## 5.2. Gas Chromatographic Analysis

- 5.2.1. Inject 5  $\mu$ l sample.
- 5.2.2. If resulting chromatogram is off scale, dilute sample with hexane until a 5  $\mu$ l aliquot gives a chromatogram with a 50% full scale deflection.
- 5.2.3. Inspect chromatogram and determine which PCB if any is present.
- 5.2.4. Inject 5  $\mu$ l corresponding PCB standard and compare to that of sample.
- 5.2.5. If sample and standard correspond, calibrate instrument by injecting a 3  $\mu$ l and 2  $\mu$ l aliquot of standard.

## 6. Calculation and Reporting

- 6.1. Plot a calibration curve using total peak height of the major compounds equivalent to 2, 3 and 5 ng of PCB (injections of 2, 3 and 5  $\mu$ l).
- 6.2. Measure corresponding peak heights in the sample and read PCB value from graph. Calculate PCB concentration using the following formula:

$$\text{ppm PCB} = \frac{W_1 \times V_2}{V_1 \times W_2}$$

Where:

- $W_1$  = ng PCB from graph  
 $V_1$  = volume of extract injected ( $\mu$ l)  
 $W_2$  = weight of sample (gm)  
 $V_2$  = total volume of diluted sample (ml)

## 7. Precision and Accuracy

The overall recovery of PCB from oil using these procedures has been in the 90-100% range for samples containing up to 1% PCB. If higher concentrations are suspect, dilute the 20 mg sample with iso-octane to a suitable volume and from this, apply the 1 ml aliquot to the silica gel column.

## 8. Bibliography

1. U.S. Environmental Protection Agency. 1976. Analysis of Pesticide Residues in Human and Environmental Samples. Environmental Toxicology Div., Health Effects Laboratory, Research Triangle Park, N.C.
2. U.S. Department of Health Education and Welfare. 1965. Guide to the Analysis of Pesticide Residues - Volumes 1 and 2. Public Health Service, Bureau of State Services (Environmental Health), Office of Pesticides, Washington, D.C.



## THE DETERMINATION OF ORGANOPHOSPHORUS PESTICIDES

Organophosphorus pesticides (OP's) are replacing the more persistent chlorinated pesticides, especially since the ban on DDT and other organochlorinated pesticides, for the control of various pests some of which have become resistant to chlorinated pesticides. The presence of OP's in the environment results primarily from their insecticidal uses. However, due to their rapid degradation by hydrolysis or metabolic breakdown, occurrence of OP's would be expected to be sporadic. There is no indication that OP's significantly contaminate waterways even in agricultural areas where OP's are extensively used.

The mammalian toxicity of OP's is generally high but these compounds are not considered hazardous because of their rapid degradation to mostly non-toxic compounds.

Maximum permissible levels of these compounds in drinking water, food and some fish have been (or are being) set.

### Sample Handling and Preservation

#### Water

Samples must be collected in one liter brown glass bottles with Teflon or aluminum foil lined caps, and filled to the mark (800 ml). These bottles are available from Laboratory Stores and are labelled, "For PCB and Pesticide Analysis only" and have been especially cleaned for pesticide sampling. The bottles must not be over-filled because extraction is performed directly in the submitted sample bottles, due to pesticide adsorption onto the container walls. The sample should be refrigerated immediately at 2 - 5°C and remain refrigerated until ready for analysis. To prevent further degradation of OP's, 100 ml of dichloromethane (re-distilled in glass, obtained from Caledon Laboratories) should be added immediately after sampling followed by thorough shaking of bottle contents. Minimum sample size required is 800 ml.

### Selection of Method

Many techniques have been used for OP analysis, but gas chromatography (GC) has been the preferred procedure. A variety of detectors can be used such as the alkali-flame ionization detector (AFID) which is very sensitive but non-specific. The flame photometric detector (FPD) which has been the preferred detector for several years, is specific to phosphorus and/or sulphur-containing compounds. A recently developed nitrogen and phosphorus sensitive flame ionization detector (NP) is now being used for confirmation purposes. Some OP's which are not amenable to GC analysis can be analyzed by High-Performance-Liquid-Chromatography (HPLC) (not described here).

Clean-up is not usually required for water samples.

## DETERMINATION OF ORGANOPHOSPHORUS PESTICIDES

### Gas Chromatographic Method A

#### SUMMARY

<b>Matrix.</b>	Water.
<b>Substances determined.</b>	Dichlorvos, mevinphos (Phosdrin), phorate (Thimet), ronnel (fen-chlorphos), diazinon, methyl-parathion, malathion, parathion, eth-ion, methyl-trithion, chlorpyriphos (Dursban), chlorpyriphos-methyl (Reldan), disyston, fenitrothion.
<b>Interpretation of results.</b>	Results are reported in ng/ml as quantitated by comparing peak heights on gas chromatograms. Levels found are generally low except in cases of exceptional agricultural run-off.
<b>Principle of method.</b>	The OP's are solvent-extracted, the extracts are dried, concentrated and analyzed by gas chromatography with phosphorus-specific detectors.
<b>Time required for analysis.</b>	Under optimum conditions, 12 water samples can be analyzed in two days. Confirmation requires another day or two.
<b>Range of application.</b>	10 - 100 ng/l. Higher levels are determined by dilution.
<b>Standard deviation.</b>	Not available.
<b>Accuracy.</b>	Not available.
<b>Limit of detection.</b>	10 - 100 ng/l depending on individual compound.
<b>Interferences and shortcomings.</b>	Few interferences are encountered because of the specificity of the detectors. However, the presence of nitrogen-containing compounds may interfere with the phosphorus confirmation while large amounts of sulphur-containing compounds will interfere with FPD determination.
<b>Minimum volume of sample.</b>	800 ml.

**Preservation and  
sample container.**

Glass bottle of 1 liter capacity with screw cap lined with Teflon or aluminum foil, available from Laboratory Stores. Samples must be refrigerated and delivered for analysis immediately unless 100 ml of dichloromethane are added in the field and the sample shaken well before delivery. Refrigeration and storage in dark remain necessary.

**Safety  
considerations.**

OP's are toxic to mammals (including man) but normal levels found in water only require normal laboratory precautions. However, analysis of formulations or highly contaminated samples requires stringent safety precautions to avoid inhalation and/or skin contact. Antidote should be immediately available (atropine sulphate)

## DETERMINATION OF ORGANOPHOSPHORUS PESTICIDES

### Gas Chromatographic Method A

#### 1. Introduction

The OP's routinely analyzed in the laboratory include:

dichlorvos	parathion
mevinphos (Phosdrin)	ethion
phorate (Thimet)	methyl-trithion
ronnel (fenchlorphos)	chlorpyrifos (Dursban)
diazinon	chlorpyrifos-methyl (Reldan)
methyl-parathion	disyston
malathion	fenitrothion

The OP's are extracted with dichloromethane, the extracts are dried and concentrated for GC analysis, using flame photometric detection. An NP detector and a different column packing are used for confirmation.

#### 2. Interferences and Shortcomings

The GC detector used for OP determination (FPD) is specific for P and S containing compounds, thus eliminating many interferences. The presence of large quantities of sulphur-containing compounds may interfere. Water samples, however, do not require clean-up prior to GC analysis. N containing compounds may interfere with the confirmatory procedure used when OP's are detected by FPD.

Glassware must be scrupulously clean and must be solvent-rinsed prior to use although it has been routinely washed with acetone, detergent, water, distilled water and baked overnight at 300°C.

#### 3. Apparatus

##### 3.1. Extraction Procedure - Water

- 3.1.1. Graduated measuring cylinder, 100 ml, 1000 ml.
- 3.1.2. Rotary extraction apparatus, Norton.
- 3.1.3. 50 ml pipette, Pyrex.
- 3.1.4. 500 ml Erlenmeyer flask, Pyrex.
- 3.1.5. Glass fiber filter paper, pre-extracted.
- 3.1.6. 300 ml round-bottom flask, Pyrex, with 24/40 glass joint.
- 3.1.7. 15 ml calibrated centrifuge tube, Pyrex, with glass stopper.
- 3.1.8. Disposable pipettes, glass.
- 3.1.9. Rotary evaporator, Buchler.
- 3.1.10. Vortex-evaporator, Buchler.

### 3.2. GC Analysis

- 3.2.1. Tracor 222 Gas Chromatograph or equivalent.
- 3.2.2. Column, Pyrex, 12 ft x 2 mm I.D.
- 3.2.3. Detector, flame photometric (FPD), in the phosphorus mode.
- 3.2.4. Syringe, glass, 10  $\mu$ l.

### 3.3. GC Confirmation

- 3.3.1. Hewlett Packard, 5710A gas chromatograph, or equivalent.
- 3.3.2. Column, Pyrex, 6 ft and 2 mm I.D.
- 3.3.3. Detector, N-P flame ionization
- 3.3.4. Syringe, glass, 10  $\mu$ l.

## 4. Reagents

### 4.1. Extraction Procedure - Water

- 4.1.1. Dichloromethane, distilled in glass, residue-free.
- 4.1.2. Iso-octane (2,2,4-trimethyl pentane) glass-distilled, residue free.
- 4.1.3. Hexane, glass-distilled, residue-free.
- 4.1.4. Sodium sulphate, anhydrous, granular, pre-extracted.
- 4.1.5. Glass wool, pre-extracted.
- 4.1.6. Glass fibre filter paper.

### 4.2. GC Analysis

- 4.2.1. Column packing: 1.5% OV17 - 1.95% QF1 on Gas Chrom Q.
- 4.2.2. Acetone, distilled in glass, residue free.
- 4.2.3. Iso-octane, distilled in glass, residue free.
- 4.2.4. Nitrogen cylinder, high purity.
- 4.2.5. Air cylinder, high purity.
- 4.2.6. Oxygen cylinder, high purity.
- 4.2.7. Hydrogen cylinder, high purity.
- 4.2.8. Hexane, glass distilled, residue-free.
- 4.2.9. Standard stock solutions of each OP: 10 mg of OP in 100 ml of acetone (100  $\mu$ g/ml).
- 4.2.10. Standard OP mix: appropriate aliquots of each compound are made up to 100 ml with iso-octane to obtain concentrations as follows in  $\mu$ g/ml:

dichlorvos	3.0	methyl-parathion	0.2
mevinphos	2.0	malathion	0.15
phorate	0.05	parathion	0.1
diazinon	0.05	methyl-trithion	1.0
disyston	0.1	ethion	0.05
ronnel	0.1		

A second mix is obtained in the same way, containing:

dichlorvos	0.6	fenitrothion	0.2
chlorpyrifos-methyl	0.2	parathion	0.06
chlorpyrifos	0.6		

#### 4.3. GC confirmation

- 4.3.1. Column packing: 6% Carbowax on Chromosorb W.
- 4.3.2. Same as in 4.2.2, 4.2.3, 4.2.5, 4.2.7, 4.2.8, 4.2.9, 4.2.10.
- 4.3.3. Helium cylinder, high purity

### 5. Procedure

#### 5.1. Extraction - Water

- 5.1.1. Add 100 ml of dichloromethane to sample, if this was not done in the field.
- 5.1.2. Place bottle on rotary extractor.
- 5.1.3. Rotate for 20 minutes: ascertain that mixing of phases is thorough.
- 5.1.4. Remove bottle from extractor and allow to settle.
- 5.1.5. Using 50 ml pipette, withdraw all the dichloromethane from the sample bottle and carefully transfer to a 500 ml conical flask (Erlenmeyer).
- 5.1.6. Repeat this sequence twice more using two 50 ml portions of dichloromethane.
- 5.1.7. When all the dichloromethane has been transferred to the conical flask, add sufficient sodium sulphate (anhydrous) to adsorb the visible water present in the extract.
- 5.1.8. Let stand for half an hour to ensure complete adsorption of water.
- 5.1.9. Prepare filter funnel by inserting glass-fiber filter paper and adding about 5 g of sodium sulphate. Rinse with dichloromethane.
- 5.1.10. Filter the combined dried dichloromethane extracts through the sodium sulphate in the filter funnel into a 300 ml round-bottom distilling flask.
- 5.1.11. Rinse the conical flask with two 10 ml portions of dichloromethane, filter into the distilling flask.
- 5.1.12. Rinse the sodium sulphate in the filter funnel with two 2 - 4 ml portion of dichloromethane.

- 5.1.13. Add about 3 ml of iso-octane to the extract as a keeper, to prevent losses of organophosphates during evaporation.
- 5.1.14. Rotary evaporate the dichloromethane extract at room temperature; adjust the rate of condensation to about one drop per second; evaporate down to near dryness (0.1 ml or so).
- 5.1.15. Quantitatively, transfer extract to 15 ml centrifuge tube, and make up (or blow down) to 1 ml with iso-octane.
- 5.1.16. Submit for gas chromatographic analysis.
- 5.1.17. Measure volume of water sample, using a 1000 ml graduated cylinder.

## 5.2. GC Analysis

- 5.2.1. Set up the following operating conditions for FPD and column packing (4.2.1.).

Injector: 300°C

Detector: 170°C

Column oven initial: 180°C

final: 250°C, hold for 10 min.

programme rate: 4 degrees/minute

Gas flows:

rotometer settings: Hydrogen: 150 at 26 psi cylinder pressure

Air: 25 at 38 psi cylinder pressure

Oxygen: 20 at 20 psi cylinder pressure

Nitrogen: 25 at 68 psi cylinder pressure

### 5.2.2. Determination of OP's

- 5.2.2.1. Turn on chromatograph and carrier gas flows. Let carrier gas flow through column at room temperature for at least 20 minutes prior to raising column oven temperature. This allows for the purging of oxygen from the packing. Allow 2-3 hours for conditions to stabilize after setting column oven at 250°C, injector at 300°C.

**NOTE:** It is normal to leave the chromatograph running continuously so as to avoid these long stabilization periods and prevent changes in column performance and detector response.

- 5.2.2.2. Carefully light detector flame by turning off hydrogen, purging for 15 seconds and igniting while slowly increasing hydrogen flow.

**NOTE:** Rapid increase in hydrogen flow may cause "explosion" and damage the detector.

- 5.2.2.3. Cool oven to initial temperature (180°C) and allow equilibration time of 5 minutes.
- 5.2.2.4. Verify the reproducibility of the detector response by injecting 2 or more identical aliquots of a standard OP mix until the response stabilizes ( $\pm 2\%$  on consecutive injections).

**NOTE:** If any problems are encountered with response baseline, spurious peaks, noise, etc., these must be eliminated before proceeding further (see trouble shooting instructions in Instruction Manual).

5.2.3. Run samples by injecting 5  $\mu$ l of each extract (5.1.15).

5.2.4. If OP's are present proceed to GC confirmation.

### 5.3. GC Confirmation

5.3.1. Operating conditions:

Injector: 250°C

Detector: 300°C

Column oven initial: 160°C

final: 240°C, hold for 8 min.

programme rate: 4 degrees/minute

Gas flows: Helium carrier: 30 ml/min

Hydrogen: 30 ml/min

Air: 40 ml/min.

5.3.2. Turn on chromatograph and allow to equilibrate as described in 5.2.2.1.

5.3.3. Ignite flame of detector and adjust voltage across detector for optimum sensitivity (see GC instruction manual).

5.3.4. Cool oven to initial temperature (180°C) and allow equilibration time of 5 minutes.

5.3.5. Verify the reproducibility of the detector response by injecting 2 or more identical aliquots of a standard OP mix until the response stabilizes ( $\pm$  2% on consecutive injections).

**NOTE:** If any problems are encountered with response, baseline, spurious peaks, noise, etc., these must be eliminated before proceeding further (see trouble shooting instructions in Instruction Manual).

5.3.6. Run samples by injecting 5  $\mu$ l of each.

5.3.7. Compare retention times, if they correspond to same standards as in 5.2.5 and if quantitative results agree, the OP identification is complete.

## 6. Calculation and Reporting

6.1. Plot a calibration curve using the peak heights of the relevant compounds equivalent to 2, 3 and 5  $\mu$ l of OP standard.

Measure corresponding peak heights in sample and read the OP value from the graph.



- 6.2. Calculate OP concentration using the following formula. Results are expressed as ppb OP.

$$\text{ppb OP} = \frac{W_1 \times V_2}{V_1 \times V}$$

Where:

$W_1$  = ng OP from graph

$V_1$  = volume of extract injected x 1000

$V_2$  = final volume of extract (ml)

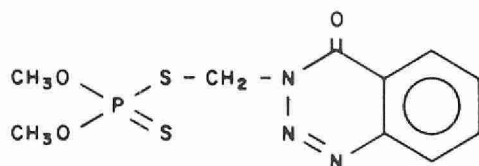
$V$  = initial volume of sample (800 ml)

## 7. Precision and Accuracy

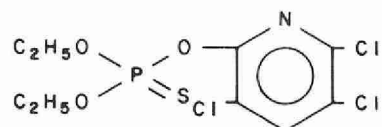
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## 8. Bibliography

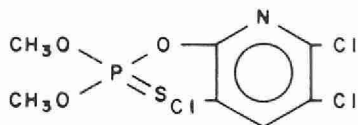
- 1.) U.S. Environmental Protection Agency. 1976. Analysis of Pesticide Residues in Human and Environmental Samples. Environmental Toxicology Div., Health Effects Laboratory, Research Triangle Park, N.C.
- 2.) U.S. Department of Health, Education and Welfare. 1965. Guide to the Analysis of Pesticide Residues - Volumes 1 and 2. Public Health Service, Bureau of State Services (Environmental Health), Office of Pesticides, Washington, D.C.



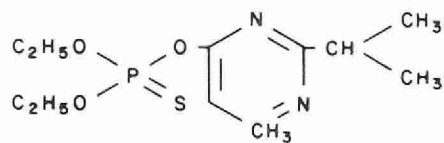
(1) AZINPHOS-METHYL



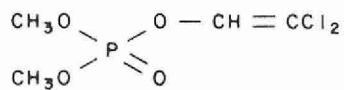
(2) CHLORPYRIPHOS



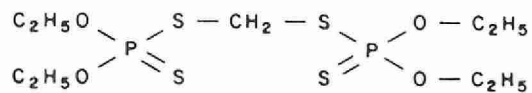
(3) CHLORPYRIPHOS-METHYL



(4) DIAZINON

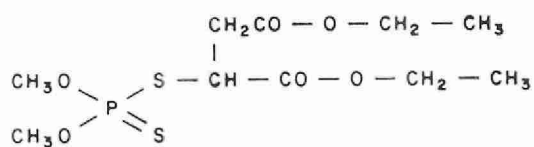


(5) DICHLORVOS

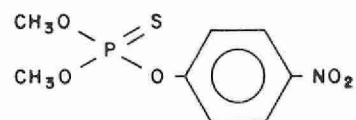


(6) ETHION

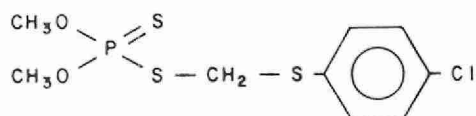
FIGURE 1 - ORGANOPHOSPHORUS PESTICIDE STRUCTURES



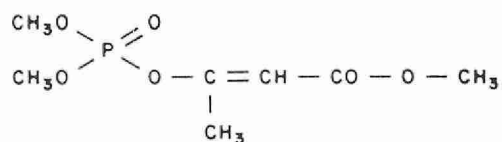
(7) MALATHION



(8) METHYL-PARATHION

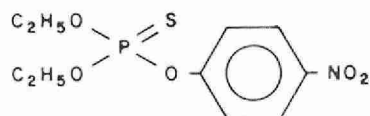


(9) METHYL-TRITHION

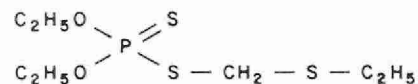


EXISTS IN CIS- & TRANS-ISOMERS; IN THE MODERN PRODUCT THE  $\alpha$  OR CIS-ISOMER IS 64.5% OF THE PRODUCT.

(10) MEVINPHOS



(11) PARATHION



(12) PHORATE

FIGURE 2 - ORGANOPHOSPHORUS PESTICIDE STRUCTURES

## **THE DETERMINATION OF OXYGEN DEMAND - BIOCHEMICAL**

The biochemical oxygen demand ( $BOD_5$ ) is a measure of the dissolved oxygen required for the stabilization of organic wastes by aerobic bacteriological and chemical oxidation. The  $BOD_5$  is used to assess the oxygen demand of organic wastes to determine the biodegradable loadings to treatment plants, and to evaluate the efficiency of waste treatments.

The test requirement for a five day incubation period imposes a lengthy time delay in establishing the demand of a particular waste. When prompt response is important, the COD test can prove valuable provided that comparison data for this test with  $BOD_5$  is available for the particular source of waste. COD tests can be completed within 24 hours.

### **Sample Handling and Preservation**

Samples can be collected in plastic or glass bottles, but must be refrigerated and stored in the dark to minimize bacterial and photosynthetic activity. Preservatives are not employed lest they retard bacterial action during incubation.

### **Selection of Method**

For  $BOD_5$  measurements in water, sewage, and industrial waste, a modification of the technique described by McGowan, Frye and Kershaw is used. The  $BOD_5$  is a measure of the dissolved oxygen depletion during a 5-day incubation period at a specified temperature, ( $20^{\circ}\text{C}$ ); it is reported in mg BOD per liter of sample. Modifications are introduced, if necessary, to ensure a suitable level of bacterial activity.

## OXYGEN DEMAND - BIOCHEMICAL

### Incubation Method

#### SUMMARY

<b>Matrix</b>	This method is used routinely on water, sewage and industrial waste.
<b>Substance determined.</b>	BOD is the amount of dissolved oxygen in mg/l required for the stabilization of organic wastes by aerobic bacteriological and chemical oxidation.
<b>Interpretation of results.</b>	The BOD results are used for assessing the oxygen demand of organic wastes and their potential effects on the aquatic environment. BOD data are also used in measuring biodegradable waste loadings to treatment plants and in evaluating the efficiency of such treatment systems.
<b>Principle of method.</b>	Dissolved oxygen determinations are performed according to OXYGEN DISSOLVED METHOD B on either a diluted or undiluted sample before and after a 5 day incubation period at 20°C. The difference between these determinations is a measure of the oxygen depletion caused by the bacteriological oxidation of organic wastes in the sample aliquot.
<b>Time required for analysis.</b>	Approximately 50 samples can be set up in one day by one person.
<b>Range of application.</b>	0.2 to 6 mg/l BOD <sub>5</sub> on undiluted surface water, sewage and industrial waste samples. Samples with higher BOD <sub>5</sub> values may be determined by using an appropriate dilution. A sample dilution giving 50% oxygen depletion over the test period is preferred.
<b>Standard deviation.</b>	The average standard deviation for rivers and lakes is 0.10 mg/l BOD (<20 mg/l range) while that for sewages and industrial wastes is 5.31 mg/l BOD (100-500 mg/l range).
<b>Accuracy.</b>	Recovery of a mixed glucose-glutamic acid standard is 88.9% of the expected value (8.2).
<b>Detection criteria.</b>	0.1 for the 0 - 20 mg/l BOD range; 1.30 for the 0 - 100 mg/l BOD range.

**Interferences and shortcomings.**

Residual chlorine, phenols, cresols, sulfides, cyanides, heavy metals and adverse pH conditions are commonly toxic to microbial populations. Sample pretreatment to adjust the pH and remove residual chlorine is routinely performed. If seeding is required, care must be taken to optimize its quality and quantity.

The presence of algae can alter the BOD through photosynthetic activity. Technical problems such as supersaturation of DO, reaeration or temperature fluctuations during incubation can alter the BOD if adequate precautions are not observed.

**Minimum volume of sample.**

500 ml for river and water samples.  
150 ml for sewage and industrial waste samples.

**Preservation and sample container.**

Glass bottles (32 oz) are used as sample containers. The samples are perishable and must be stored under refrigeration and analyzed as quickly as possible. Preservatives are not used since they could retard bacterial activity during the test procedure.

**Safety considerations.**

Use hand and eye protection when dispensing concentrated acids and bases. Carefully read the labels on all industrial waste samples before proceeding with any analysis. Adequate warnings are usually provided if a possible hazard exists.

## OXYGEN DEMAND - BIOCHEMICAL

### Incubation Method

#### 1. Introduction

The biochemical oxygen demand is determined by preparing in duplicate suitable sample aliquots and making up to volume with synthetic dilution water. The dilution should be such that about 50% of the dissolved oxygen is depleted after 5 days incubation. The dissolved oxygen is determined immediately on 1 bottle and on the other bottle after the incubation period using OXYGEN DISSOLVED METHOD B. The BOD is expressed as the amount of dissolved oxygen in mg utilized by 1 liter of sample during a 5 day incubation period at 20°C, (8.1, 8.2, 8.11, 8.13, 8.20).

#### 2. Interferences and Shortcomings

##### 2.1. Physical Effects

###### 2.1.1. Reaeration

If samples are not incubated in tightly sealed bottles with no entrapped air, reaeration from the atmosphere may occur with a subsequent apparent decrease in the BOD (8.2, 8.7).

###### 2.1.2. Supersaturation of Oxygen

Samples supersaturated with oxygen may lose excess oxygen by bubble formation during the incubation period resulting in a high BOD. To prevent supersaturation of oxygen, samples should be warmed to room temperature and shaken vigorously before dilution (8.2, 8.7).

###### 2.1.3. Lack of Nutrients

Inadequate nutrients may cause a decrease in the bacterial population, decreasing the rate of biochemical oxidation and causing a low BOD results. This problem is prevalent only in undiluted samples since the dilution water used at this laboratory has nutrients added (8.2, 8.7).

###### 2.1.4. Algae

Since green algae can produce oxygen in the presence of light and carbon dioxide through photosynthetic process, samples containing algae must be protected from direct sunlight and samples are always incubated in the dark.

###### 2.1.5. Time before Analysis

Most samples contain sufficient micro organisms from their natural environment to support continuous biochemical oxidation processes.

Storage under refrigeration retards bacterial activity. A sample will not be representative of the source at the time of sampling if too much time elapses prior to analysis. Ideally the BOD test should be initiated on the day of sampling (8.2).

#### 2.1.6. Incubation Temperature

The temperature must be constant over the 5 day incubation period. A change of  $\pm 1^{\circ}\text{C}$  will cause a 5% change in BOD.

#### 2.1.7. Dilution Effect

Improper dilution will give erroneous results. The best BOD results are obtained when approximately 50% of the dissolved oxygen is depleted. As a general rule, samples which do not show a DO depletion of at least 2 mg/l or do not contain a residual DO of at least 2 mg/l after incubation should be repeated at a different dilution.

#### 2.1.8. Seeding Effects

Certain industrial wastes containing toxic substances require a specially acclimatized microbial population that is not necessarily present in domestic sewage. Acclimatization of a seed has an important influence on the rate of oxidation; thus different acclimatized seeds will produce different rates of oxidation. Comparisons with routine BOD results from common sewage samples should be avoided (8.2, 8.3, 8.4, 8.15).

### 2.2. Toxicity Effects

#### 2.2.1. pH

Optimum bacterial activity occurs from pH 6.5 to pH 8.3. Samples with a pH outside of this range must be adjusted to neutrality ( $\approx \text{pH} = 7.0$ ) with the necessary amount of acid or base and reseeded prior to analysis (8.2, 8.15).

#### 2.2.2. Germicidal Organic Compounds

Phenol, cresols or other germicidal organic compounds will kill off the microbial population, reducing the rate of biochemical oxidation, and cause a low BOD result. Only acclimatized seeds will be effective in oxidizing this type of waste (8.2, 8.3, 8.4, 8.15).

#### 2.2.3. Chlorine

Residual Chlorine is commonly used as a germicide in final effluents from sewage treatment plants. Such samples are treated with the required amount of sodium sulphite to facilitate the complete removal of residual chlorine and reseeded prior to analysis (8.2, 8.9).



#### 2.2.4. Others

Most heavy metals, sulfide, and cyanide interfere with biological processes. The calcium salt of ethylene-diaminetetraacetic acid (CaEDTA) can be used to suppress the effects of copper and zinc below 5.0 mg/l but has little effect on other metals.

The following table summarizes the various effects of toxic substances on the BOD for incubated samples (8.2, 8.9, 8.20).

Concentration of Toxic Substances in mg/l of Incubated Samples

% BOD Reduction	Chlorine	Copper	Hexavalent Chromium	Trivalent Chromium	Nickel	Zinc	Cyanide
5	0.05	0.05	0.07	0.035	0.10	0.30	0.30
10	0.10	0.10	0.20	0.69	0.24	0.60	0.60
20	0.20	0.20	3.3	1.4	1.0	1.5	1.2
30	0.27	0.30	4.3	2.1	2.9	1.9	2.7
40	0.33	0.50	5.9	3.4	4.8	2.3	4.3
50	0.40	1.0	9.7	6.1	7.3	2.6	> 5.0
60	0.48	5.0	14.0	15.0	>15.0	3.0	-
70	0.53	7.7	>15.0	15.0	-	7.5	-
80	0.60	>10.0	-	-	-	>15.0	-
90	0.63	-	-	-	-	-	-

### 2.3 Nitrification Processes

The carbonaceous oxygen demand and the nitrogenous oxygen demand are the major components contributing to the BOD. Nitrogenous material utilizes dissolved oxygen when ammonia is oxidized first to nitrite and then to nitrate by special types of nitrifying bacteria. Nitrification does not normally begin until most of the carbonaceous demand has been satisfied; therefore the contribution of nitrification to the BOD is most prevalent in effluents and stream samples which have already undergone partial oxidation. Special procedures are available for separating the carbonaceous oxygen demand from the nitrogenous oxygen demand.

## 3. Apparatus

- 3.1. Incubation bottles, approximately 174 ml capacity (Prince of Wales type) with polyseal caps.

- 3.2. Air Incubator to provide a constant temperature of  $20^{\circ}\text{C} \pm 1^{\circ}\text{C}$ .
- 3.3. pH meter.
- 3.4. Mixing Vats, plastic, 200 liter capacity (2).
- 3.5. Electric mixers (2).
- 3.6. Graduated cylinders, 100 ml (1), 250 ml (4), 500 ml (6), 1000 ml (2).
- 3.7. Erlenmeyer flasks, 150 ml, 500 ml.
- 3.8. Funnel, glass, to accommodate 14 inch filter paper.
- 3.9. Filter paper, 14 inch, cellulose. (For filtering calcium chloride reagent)
- 3.10. Filter paper 9.0 cm, glass fibre. (For filtered  $\text{BOD}_5$ )
- 3.11. Weston and Stack Oxygen Analyzer.
- 3.12. Pipettes, 5.0 ml, 10.0 ml, 25.0 ml, 50 ml, 100 ml

#### 4. Reagents

- 4.1. Acetic acid ( $\text{CH}_3\text{CO}_2\text{H}$ ) concentrated reagent grade.
- 4.2. Sulphuric acid ( $\text{H}_2\text{SO}_4$ ) concentrated reagent grade.
- 4.3. Potassium iodide (KI) reagent grade pellets.
- 4.4. Sodium hydroxide (NaOH) reagent grade pellets.
- 4.5. Sodium thiosulphate pentahydrate ( $\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$ ) reagent grade crystals.
- 4.6. Ferric chloride hexahydrate ( $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ ) reagent grade.
- 4.7. Salicylic acid ( $\text{C}_6\text{H}_4(\text{OH})\text{COOH}$ ) reagent grade crystals.
- 4.8. Calcium chloride ( $\text{CaCl}_2$ ) anhydrous mesh, reagent grade.
- 4.9. Magnesium sulphate heptahydrate ( $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ ) reagent grade crystals.
- 4.10. Potassium dihydrogen orthophosphate ( $\text{KH}_2\text{PO}_4$ ) reagent grade crystals.
- 4.11. Dipotassium hydrogen orthophosphate ( $\text{K}_2\text{HPO}_4$ ) reagent grade crystals.
- 4.12. Disodium hydrogen orthophosphate ( $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$ ) reagent grade crystals.
- 4.13. Ammonium chloride ( $\text{NH}_4\text{Cl}$ ) reagent grade crystals.
- 4.14. Starch, soluble reagent grade powder.
- 4.15. Sodium sulphite ( $\text{Na}_2\text{SO}_3$ ) reagent grade crystals.

4.16. Glucose ( $C_6H_{12}O_6$ ) reagent grade

4.17. Glutamic acid ( $NH_2CH(COOH).CH_2CH_2COOH$ ) reagent grade

**4.18. Acetic Acid (50%)**

Dilute 500 ml of concentrated acetic acid to about 1 liter with distilled water and mix thoroughly.

**4.19. Potassium Iodide Solution (5%)**

Dissolve 25 g of potassium iodide crystals in distilled water, dilute to 500 ml and mix thoroughly.

**4.20. Sodium Thiosulphate Solution (0.0125N)**

Dissolve 6.205 g sodium thiosulfate pentahydrate in freshly boiled distilled water, dilute to 2 liters with freshly boiled distilled water and mix thoroughly. Preserve with 2.0 g NaOH pellets.

**4.21. Sodium sulphite (0.01N)**

Dissolve 630 mg sodium thiosulfate to 1 liter with freshly boiled distilled water and mix thoroughly.

**4.22. Sodium Hydroxide Solution (1N)**

Dissolve 40 g sodium hydroxide pellets in distilled water, dilute to 1 liter and mix thoroughly.

**4.23. Sulphuric Acid (1N)**

Mix 28 ml concentrated sulphuric acid with distilled water, dilute to 1 liter and mix thoroughly.

**NOTE:** Use hand and eye protection.

**4.24. Calcium Chloride Stock Solution**

Dissolve 55 g of anhydrous calcium chloride in distilled water, dilute to 2 liters, mix thoroughly and filter.

**4.25. Magnesium Sulphate Stock Solution**

Dissolve 45 g of magnesium sulphate heptahydrate in distilled water, dilute to 2 liters, mix thoroughly and filter.

**4.26. Ferric Chloride Stock Solution**

Dissolve 0.50 g of ferric chloride hexahydrate in distilled water, dilute to 2 liters.

**4.27. Phosphate Buffer**

Dissolve 17 g potassium dihydrogen orthophosphate, 43.50 g dipotassium hydrogen orthophosphate, 89.2 g disodium hydrogen orthophosphate and 3.44 g ammonium chloride in distilled water, dilute to 2 liters and mix thoroughly.

#### 4.28. Dilution Water

Mix 200 ml of calcium chloride-stock solution, 200 ml of ferric chloride stock solution, 200 ml of magnesium sulphate stock solution, and 200 ml of phosphate buffer together in a plastic vat and dilute with distilled water to 200 liters. Aerate vigorously with an electric mixer for about 2 hours in an incubator at 20 °C to ensure saturation of dissolved oxygen.

#### 4.29. Seed Solution

Prepare a mixture of raw sewages using 4 or 5 different samples, each from a different treatment plant. Aerate the mixture overnight and withdraw the supernatant for use as seed.

#### 4.30. Starch Solution

Prepare a suspension of 6 g soluble starch and 1.25 g salicylic acid with approximately 100 ml distilled water and slowly add with stirring to about 800 ml of boiling distilled water. Dilute to 1 liter, allow to boil for a few minutes, let settle overnight and retain supernatant.

#### 4.31. Quality Control Solutions

Dry glucose and glutamic acid at 103 °C for one hour, and store them in a desiccator. For QC-A, dissolve 225 mg of each compound in 1 litre; store 30 ml aliquots in a freezer. For QC-B, dissolve 150 mg of each compound in 1 litre; store 30 ml aliquots in a freezer.

### 5. Procedure

#### 5.1. Choice of Aliquot

The BOD of each sample must first be estimated, taking into consideration the type of sample, colour, amount of suspended solids, and odour. Usually 2 or 3 dilutions are suggested to insure a proper BOD determination. Record these dilutions directly on the sample bottles with a waterproof marker. The ability to choose the correct dilutions for a BOD determination takes considerable experience.

#### 5.2. Pretreatment of Samples

##### 5.2.1. pH Adjustment

Samples with a pH  $\leq 6.5$  and  $\geq 8.3$  must be adjusted prior to dilution. Pour an appropriate volume of sample into a labelled pyrex beaker and adjust to approximately pH 7 with a pH meter using the necessary amount of 1N sodium hydroxide or 1N sulphuric acid. Use the neutralized sample for subsequent BOD dilutions.

##### 5.2.2. Removal of Residual Chlorine

Place 50 ml of sample, 5 ml of 5% potassium iodide solution, 5 ml of 50% acetic acid, and about 3 ml of starch solution into a 250 ml Erlenmeyer flask and mix by swirling. If residual chlorine is present in the sample, the colour will turn blue. In this case add 0.01N sodium sulphite dropwise with continuous swirling, noting the number of drops required for the colour to disappear. Record this amount as

drops per 50 ml of sample directly on the sample bottle. The required amount of sodium sulphite necessary for neutralization will later be added to portions of the original sample prior to dilution.

#### 5.2.3. Filtration

Samples submitted for filtered BOD determinations are filtered through fibre-glass filter paper prior to dilution.

### 5.3. Dilution

5.3.1. Set up a series of 8 BOD bottles (Prince of Wales type, 174 ml capacity) and label as dissolved oxygen blanks.

5.3.2. Set up 2 BOD bottles for each dilution of each sample; (in some cases dilution is not required). One bottle is analyzed for dissolved oxygen on the first day and the other analyzed for dissolved oxygen after a 5 day incubation period.

5.3.3. Mark on each bottle the appropriate sample number and the dilution required. 5% and 2% dilutions are routinely performed on the seed solution, labelling such bottles "SEED" with their respective dilution.

5.3.4. No dilutions are necessary for samples marked "100%" dilution. For samples requiring dilutions of 50%, 25%, 10% and 5%, appropriate aliquots of samples are pipetted into a graduated Erlenmeyer, diluted to the proper volume with dilution water, and mixed by swirling. For dilutions below 5%, an initial 5% dilution of the sample is prepared and appropriate aliquots of this solution are pipetted into the BOD bottles (174 ml) to obtain the assigned dilutions; the bottles are then filled up with dilution water.

**NOTE:** For samples with high or low pH values, use the previously neutralized sample and add 1 ml of seed solution per bottle.

**NOTE:** For samples containing residual chlorine, add the necessary amount of sodium sulphite and 1 ml of seed solution per bottle.

5.3.5. Prepare the 8 DO blanks by filling the corresponding BOD bottles with dilution water only. Incubate 4 bottles.

5.3.6. Tightly seal 1 set of BOD bottles with clean polyseal caps.

**NOTE:** No air bubbles should remain in the bottles.

5.3.7. Place the sealed bottles in an incubator for 5 days at 20°C.

5.3.8. Determine the dissolved oxygen in the remaining set of BOD bottles immediately using DO method B.

5.3.9. After 5 days remove the first set of BOD bottles from the incubator and determine the dissolved oxygen using DO Method B.

## 6. Calculation and Reporting

- 6.1. Calculations for the biochemical oxygen demand are determined using the following equations. Special corrections must be included for seeded samples.

Let  $DO_0$  = Dissolved oxygen concentration in diluted samples prior to incubation.

$DO_5$  = Dissolved oxygen concentration in diluted samples after 5 day incubation period.

$d$  = Percent dilution of sample.

$DO_{s0}$  = Dissolved oxygen concentration in diluted seed solution prior to incubation.

$DO_{s5}$  = Dissolved oxygen concentration in diluted seed solution after 5 day incubation period.

$f$  = Seed dilution ratio.

Where:

$$f = \frac{\text{Percentage of Seed solution in } DO_0}{\text{Percentage of Seed solution in } DO_{s0}}$$

when seed solution is not used:

$$\text{mg/l} = \frac{DO_0 - DO_5}{d} \times 100$$

when seed solution is used:

$$\text{mg/l BOD} = \frac{(DO_0 - DO_5) - (DO_{s0} - DO_{s5}) f}{d} \times 100$$

The incubated DO blanks are used to monitor the behaviour of the dilution water, however they are not used in the actual BOD calculations. Small depletions of dissolved oxygen regularly occur in the DO blanks during incubation (approximately 0.1 - 0.3 mg/l DO) but these depletions would tend to over-correct for oxygen loss if included in the BOD calculations.

When 2 or more dilutions are performed on the same sample, the sample dilution which gives a dissolved oxygen depletion closest to 50% is used for calculating the reported BOD result.

- 6.2. Final results are rounded off and reported to 2 significant figures according to the following tables.

### Sewage and Industrial Wastes

mg/l BOD	Report to Nearest (mg/l BOD)
0 - 2	0.2
2 - 10	0.5
10 - 20	1
20 - 50	2
50 - 100	5
100 - 200	10
200 - 500	20
500 - 1000	50
1000 - 2000	100
2000 - 5000	200
5000 - 10000	500

### River and Lake Samples

mg/l BOD	Report to Nearest (mg/l BOD)
0 - 5.0	0.2
5.0 - 10.0	0.5
10 - 50	2.0
50 - 100	5.0
100 - 1000	10

## 7. Precision and Accuracy

Based on duplicate samples, the standard deviation for the less than 20 mg/l BOD range, as found in river and lake samples, is 0.102. For sewage and industrial waste samples in the 0 - 100 mg/l BOD range the standard deviations are 0.79 in the 0 - 20 mg/l range, 2.03 in the 20 - 50 mg/l range and 3.84 in the 50 - 100 mg/l range. For sewage and industrial wastes with BOD levels from 0 - 500 mg/l the standard deviations are 3.18 in the 0 - 100 mg/l range, 6.07 in the 100 - 250 mg/l range and 6.22 in the 250 - 500 mg/l range. There is no standard against which the accuracy of the BOD test can be measured. However, the expected percentage recovery of a mixed standard is approximately 88.9% for river and lake samples and 98.2% for sewage and industrial wastes.

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## THE DETERMINATION OF OXYGEN DEMAND - CHEMICAL

Chemical oxygen demand (COD) test attempts to measure all the oxidizable organic carbon in a sample. The degree of oxidation depends on the testing conditions.

If the ratio between the chemically oxidizable and the biologically oxidizable organic fractions are constant over time then the COD test may be used to replace the BOD test. This condition is rarely attained. A correlation can sometimes be obtained in a specific waste stream from some industrial processes. The COD test is quicker than the BOD test and is therefore, applied in cases where the monitoring time of particular waste is critical.

### Sample Handling and Preservation

#### Water, Sewage and Industrial Waste

Samples should be refrigerated to minimize bacteriological activity and tested immediately. Where delays are unavoidable, the sample should be preserved by acidification with sulphuric acid to a pH range of 3 to 5.

### Selection of Method

Moore's procedure (8.6) was chosen for COD determinations because it is applicable to a wide range of samples and superior in oxidizability to other methods. Many modifications of the original technique have been applied. Less stringent conditions and other modifications usually result in lower recovery.

## OXYGEN DEMAND - CHEMICAL

### Dichromate Method A

#### SUMMARY

<b>Matrix.</b>	This method is routinely used on water, sewage and industrial waste samples.
<b>Substance determined.</b>	Oxygen demand in mg/l of that portion of the chemical constituents in a sample which are oxidized under the conditions of the test.
<b>Interpretation of results.</b>	Results are reported as mg/l COD. The COD results may not be correlated with BOD results except in specific cases. Where such a correlation has been established, the COD may be used to replace the BOD test.
<b>Principle of method.</b>	An aliquot of sample is refluxed with concentrated sulphuric acid and dichromate ion in the presence of silver sulphate as catalyst. The excess dichromate is then titrated with standardized ferrous ammonium sulphate solution using ferroin as indicator. The amount of dichromate reduced is a measure of the chemical oxygen demand.
<b>Time required for analysis.</b>	A single analysis requires approximately 3½ hrs. By analyzing several samples concurrently, 20 samples may be tested in one day.
<b>Range of application.</b>	Using a 10 ml aliquot, COD concentrations of 3-1000 mg/l are measured on surface water samples and 6-1000 mg/l on sewage and industrial wastes. Smaller aliquots or predilution of the sample are routinely used to determine higher COD concentrations.
<b>Standard deviation.</b>	1.65 in the 3-200 mg/l COD range of river and lake samples. For sewage and industrial waste, the standard deviation is 3.35 in the 6 - 200 mg/l range, 5.32 in the 200 - 500 mg/l range and 7.30 in the 500 - 1000 mg/l range.
<b>Accuracy.</b>	The average recovery of quality control standards is 99.9% with relative standard deviations ranging from 0.64 to 1.59%.
<b>Detection criteria.</b>	2.71 mg/l COD.
<b>Interferences and shortcomings.</b>	Straight chain aliphatic compounds, pyridine and aromatic hydrocarbon are not completely oxidized. The addition of silver sulphate as a catalyst increases the oxidation of straight chain compounds (8.1).

Chloride interference is suppressed by the addition of mercuric sulphate to the reflux mixture. Nitrite-N interference is disregarded due to the generally low concentrations (less than 1 mg/l) encountered, but may be eliminated by adding sulphamic acid to the reflux mixture.

**Minimum volume  
of sample.**

25 ml.

**Preservation and  
sample container.**

Glass bottles are recommended. Refrigerated shipment and storage followed by immediate testing is preferred. Where delays are unavoidable, the sample may be preserved by adjusting the pH to between 3 and 5 with sulphuric acid.

**Safety  
considerations.**

Large quantities of concentrated sulphuric acid are used in this method. Adequate care must be taken to ensure: safe dispensing of the acid; complete mixing of the reflux mixture before heating in order to prevent the mixture from blowing out of the flask; cooling of the reflux mixture before condenser removal; and proper disposal of the acid waste.

EYE PROTECTION MUST BE WORN WHEN HANDLING THE CONCENTRATED ACID AND WHEN REFLUXING.

## OXYGEN DEMAND - CHEMICAL

### Dichromate Method A

#### 1. Introduction

Organic matter in an aliquot of sample is oxidized as completely as possible by refluxing for 2 hours with a known quantity of standard potassium dichromate and mercuric and silver sulphates in 50% sulphuric acid. The sample aliquot is chosen so that the amount of dichromate is always in excess of the amount required for oxidation. The unreacted dichromate is titrated with standardized ferrous ammonium sulphate using ferroin as the indicator. A reagent blank is prepared and analyzed by the same procedure. The difference between the titration volumes of the refluxed blank and the sample solution yields the depletion of dichromate due to the oxidation of the organic matter in the aliquot.

#### 2. Interferences and Shortcomings

One gram of chloride ion exerts a COD equivalent of 226 mg/l. This interference can be suppressed by the addition of 0.2 g of mercuric sulphate to the reflux mixture which will tie up the chloride as a soluble mercuric chloride complex (8.1). This amount of mercuric sulphate is sufficient to treat a 10 ml aliquot containing 2000 mg/l of chloride ion.

One gram of nitrite-N exerts an equivalent of 1140 mg/l of COD. Since the concentrations of nitrite-N encountered are generally low, this interference can be ignored. However, if required, an addition of a 10:1 ratio of sulphamic acid: nitrite-N will eliminate the nitrite-N interference.

Oxidation efficiency for most organic compounds varies from 85% to 100%. Efficiency increases markedly as the number of branch chains and substituent active groups (i.e. hydroxyl, carboxyl, keto, amino) increases. Five membered rings (e.g. pyridine) are incompletely oxidized. Recovery of ammonia and nitrogen in amines is insignificant.

#### 3. Apparatus

- 3.1 Boiling flask, 100 ml, round bottom,  $\text{\text{F}}$  24/40 outer joint. (24)
- 3.2 Friedrichs condensers,  $\text{\text{F}}$  24/40 inner joint on lower end. (24)
- 3.3 Hot plate or heating rack, capable of 9 watts/square inch of heating surface with supporting racks for condensers and flasks during refluxing.
- 3.4 Burette, 5.0 ml (0.01 ml divisions) auto-zero pressure fill type.
- 3.5 Burette stand, lighted frame and base type preferred.

- 3.6. Magnetic stirrers and 1 inch (2.5 cm) stirrer bars. (12)
- 3.7. Reagent bottles and bulk storage.
- 3.8. Anti-bumping granules.

**NOTE:** All glassware must be kept scrupulously free of organic matter; otherwise gross errors in COD will result. Dichromic acid is recommended for cleaning of the boiling flasks and reagent bottles, and any such cleansing must be followed by very thorough rinsing. Appropriate safety precautions must be observed when handling dichromic acid, a very dangerous and corrosive reagent.

#### 4. Reagents

- 4.1. Potassium dichromate ( $K_2Cr_2O_7$ ) reagent grade crystals.
- 4.2. Ferrous ammonium sulphate hexahydrate ( $Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$ ) reagent grade crystals.
- 4.3. Sulphuric acid ( $H_2SO_4$ ), concentrated, reagent grade.
- 4.4. Silver sulphate ( $Ag_2SO_4$ ) reagent grade powder.
- 4.5. Mercuric sulphate ( $HgSO_4$ ) reagent grade powder.

**NOTE:** POISONOUS WHEN DISPENSING. DO NOT ALLOW THIS POWDER TO BECOME AIRBORNE.

- 4.6. Ferrous sulphate ( $FeSO_4 \cdot 7H_2O$ ), reagent grade powder.
- 4.7. 1, 10-phenanthroline monohydrate, reagent grade crystals.
- 4.8. Potassium hydrogen phthalate ( $COOH C_6H_4 COOK$ ) reagent grade crystals.
- 4.9. **Standard Potassium Dichromate Solution (0.25 N)**

Dry a sufficient quantity of potassium dichromate, reagent grade crystals, at 103 C for 2 hrs. Cool in a desiccator. In a 1 liter volumetric flask dissolve 12.25 g of the dried potassium dichromate in distilled water. Dilute to 1.0 liter with distilled water and mix well. Transfer to a bulk reagent storage bottle.

#### 4.10. Sulfuric Acid - Silver Sulphate Reagent

Add 22 g silver sulphate to a 9 lb bottle of concentrated sulphuric acid. Allow 1 to 2 days for complete dissolution. Mix well before use. If this reagent is stored in a clear glass bottle, keep the solution in darkness.

Safety precautions must be observed in handling concentrated sulphuric acid, a very corrosive chemical.

#### 4.11. Ferroin Indicator

Dissolve 2.970 g 1,10-phenanthroline monohydrate and 1.390 g ferrous ammonium sulphate hexahydrate in distilled water and dilute to 200 ml.

#### 4.12. Standard Ferrous Ammonium Sulphate (FAS) Solution (0.25 N)

In a 2 liter volumetric flask dissolve 196 g analytical grade crystals of ferrous ammonium sulphate hexahydrate in approximately 1 liter distilled water. While stirring cautiously add 40 ml concentrated sulphuric acid. Dilute to the 2 liter mark with distilled water and mix well. This solution must be standardized daily.

##### Standardization

Pipette 5.00 ml standard potassium dichromate solution into a 100 ml Erlenmeyer flask and add 10 ml distilled water. Cautiously add 15 ml sulphuric acid, mix well and cool to room temperature.

**NOTE:** WEAR EYE PROTECTION.

Add exactly 2 drops of ferroin indicator and titrate with 0.25 N FAS solution. Record the volume of titrant.

#### 4.13. Standard Check (500 mg/l) (Rivers and Lakes Samples)

This solution is prepared in the same way as in QC-B 4.14 and is used as a check on the system.

#### 4.14. Quality Control Solutions (Sewage and Industrial Waste)

QC-A: Dissolve 0.8502 g potassium hydrogen phthalate in distilled water and dilute to 1 liter. Preserve with 0.2 g mercuric sulphate. This solution has a COD of 1000 mg/l.

QC-B: Dissolve 0.4251 g potassium hydrogen phthalate in distilled water and dilute to 1 liter. Preserve with 0.2 g mercuric sulphate. This solution has a COD of 500 mg/l.

#### 4.15. Quality Control Solutions (Rivers and Lakes)

QC-A: Dissolve 0.1700 g potassium hydrogen phthalate in distilled water and dilute to 1 liter. Preserve with 0.2 g mercuric sulphate. This solution has a COD of 200 mg/l.

QC-B: Dissolve 0.0850 g potassium hydrogen phthalate in distilled water and dilute to 1 liter. Preserve with 0.2 g mercuric sulphate. This solution has a COD of 100 mg/l.

### 5. Procedure

5.1. With each batch of samples, include one blank, QC-A, QC-B, and one duplicate.

5.2. Place 4-6 anti-bumping granules and approximately 0.2 g (on the end of a spatula) of mercuric sulphate into a 100 ml boiling flask.

**NOTE:** Do not allow mercuric sulphate powder to become airborne.

5.3. Pipette 5.00 ml of 0.25N potassium dichromate solution into the flask.

5.4. Mix sample well. Pipette, using a wide mouth pipette, 10.0 ml or a suitably smaller aliquot of sample into the flask.

**NOTE:** The aliquot selected should be sufficient to yield an FAS solution titration of 1 to 4 ml upon completion of the reaction. If an aliquot of between 5 and 10 ml is taken, dilute to 10 ml with distilled water. Where aliquots of less than 5 ml are required, a 10.0 ml aliquot is taken and is prediluted in a volumetric flask so that a 10.0 ml aliquot of the dilution will yield the required amount of sample.

- 5.5. While swirling, cautiously add 15 ml of the sulphuric acid-silver sulphate solution and mix well.

**NOTE:** WEAR EYE PROTECTION. THOROUGH MIXING IS ESSENTIAL. IF THE CONTENTS OF THE FLASK ARE NOT MIXED, LOCALIZED HEATING WILL OCCUR AT THE BOTTOM OF THE FLASK RESULTING IN SEVERE BUMPING WHICH MAY BLOW THE REACTION MIXTURE OUT OF THE CONDENSER.

- 5.6. At this point the reaction mixture should turn a dark orange-brown color. If the reaction mixture remains a bright orange color, and if an aliquot of less than 10 ml has been used, it must be discarded and steps 5.1 through 5.4 repeated with a larger aliquot. If the reaction mixture turns blue-green, the contents of the flask must be discarded and steps 5.1 through 5.4 repeated with a smaller aliquot. If the reaction mixture turns cloudy, add another 0.2 g of mercuric sulphate. Following settling continue adding until further cloudiness does not occur.

- 5.7. Attach the flask to a reflux condenser and reflux for 2 hours after initial boiling occurs.

**NOTE:** Do not use grease on the ground glass joints.

- 5.8. Allow the solution to stand until cool. Wash the inside walls of the condenser by directing a strong stream of distilled water through the open end of the condenser. Allow the washings to drain into the flask.

- 5.9. Remove the flask from the reflux apparatus, mix well, and let cool to room temperature. The total volume should be approximately 45 ml.

- 5.10. Add exactly 2 drops of ferroin indicator and a magnetic stirring bar. Titrate with standardized 0.25 N FAS solution; the end point is the sharp color change from blue-green to reddish-brown, although the blue-green colour may re-develop in minutes. Record the volume of FAS required to the nearest 0.01 ml.

**NOTE:** If the difference between the titration volumes of the blank and the dichromate standard is greater than 0.1 ml, check for contaminated sulphuric acid-silver sulphate solution or a contaminated flask by running at least 2 more blanks with the same reagents. Do not report any results until the source of the contamination is identified, and eliminated or controlled. Confirm that values for QC-A and QC-B meet test specifications. If not, do not report data.

## 6. Calculation and Reporting

6.1. 
$$\text{mg/l COD} = \frac{(a-b) \times N \times \text{ml dichromate} \times 8000}{V \times C}$$



$$= \frac{(a-b) 0.25 \times 5.0 \times 8,000}{v \times c}$$

$$= \frac{(a-b) \times 10,000}{v \times c}$$

Where: a = volume FAS required to titrate blank  
 b = volume FAS required to titrate sample  
 c = volume FAS required for standardization  
 v = sample volume  
 N = normality of the dichromate  
 8000 = equivalent weight of oxygen in mg

6.2. Results are reported to three significant figures if feasible.

## 7. Precision and Accuracy

Precision is established by analyzing routine samples in duplicate:

Sample Type	Sample Concentration (mg/l COD)	Standard Deviation (mg/l COD)
Rivers and Lakes	<200	1.65
Sewage and Industrial Wastes	<200 200-500 500-1000	3.35 5.32 7.30

Accuracy is determined by analyzing quality control standards:

Sample Type	QC Concentration (mg/l COD)	Average Recovery of QC Standard (mg/l COD)
Rivers and Lakes	100	103.1
	200	198.4
Sewage and Industrial Wastes	500	501.3
	1000	996.9

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## THE DETERMINATION OF OXYGEN - DISSOLVED

An adequate supply of dissolved oxygen (DO) is required for maintaining self-purification processes in natural water systems and waste treatment plants. DO data determined in the field provides a rapid means of assessing the effect of oxidizable wastes on receiving waters, the efficiency of waste treatment during bio-chemical oxidation and the capacity of a natural body of water for maintaining aquatic life.

The test measures molecular oxygen as milligrams of oxygen per liter of solution, and is used primarily as part of the procedure for determining the biochemical oxygen demand (BOD).

### **Sample Handling and Preservation**

#### Water, Sewage and Industrial Waste

Ideally, DO measurements should be made at the sampling site. The effects of temperature and pressure, bacteriological activity, high organic content and time in transit could significantly change the DO concentration when the sample is transported. Should it not be possible to perform the test in the field, samples must be collected in glass bottles, filled completely (avoiding entrapped air), tightly sealed, refrigerated to minimize bacteriological activity and analyzed immediately upon arrival at the laboratory. Samples containing algae must be protected from sunlight in order to prevent the photosynthetic process, resulting in oxygen production. If the Winkler Method is used, the sample should be preserved by refrigeration, following acidification to a pH of less than 3 with concentrated sulphuric acid.

### **Selection of Method**

Two methods are currently employed for dissolved oxygen determinations. Method A, the Winkler Method is a classical chemical technique using a standard titration procedure. The variety of samples that can be analyzed using the Winkler method is somewhat limited due to the number of possible interferences which are not easily suppressed.

Method B, the oxygen electrode method employs a selective gas permeable membrane. Oxygen, diffusing through the membrane, is reduced at a platinum electrode (cathode), generating a current proportional to the dissolved oxygen in the sample. This method avoids the interferences of method A without the use of suppressants. Other gases which permeate through the membrane and which have reduction potentials close to that of oxygen may interfere.

## OXYGEN - DISSOLVED

### Winkler Method A

#### SUMMARY

<b>Matrix.</b>	This method is used primarily for the standardization of the oxygen electrode system. However, it may be used for DO determinations on water, sewage and industrial waste samples.
<b>Substance determined.</b>	Dissolved oxygen.
<b>Interpretation of results.</b>	Results are reported as mg/l dissolved oxygen.
<b>Principle of method.</b>	DO is determined by chemically converting any dissolved oxygen into a free iodine equivalent which is titrated with sodium thiosulphate. The calculation has been simplified such that the titrated volume of sodium thiosulphate in milliliters also reads as mg/l of DO.
<b>Time required for analysis.</b>	A single analysis requires about 10 minutes.
<b>Range of application.</b>	0.1 - 15.0 mg/l of DO. Levels above 10.0 mg/l are very rare. The maximum possible DO level varies depending on the solubility of oxygen and degree of supersaturation, e.g. air saturated distilled water at 0°C contains 14.6 mg/l of DO.
<b>Standard deviation.</b>	Not yet determined.
<b>Accuracy.</b>	Not yet determined.
<b>Detection criteria.</b>	Not yet determined.
<b>Interferences and shortcomings.</b>	Nitrite interference is suppressed by the addition of sodium azide. Ferrous iron interference is suppressed by oxidation with potassium permanganate. At low pH, ferric iron interference is removed by the addition of potassium fluoride. Turbid and colored samples containing organic carbon in excess of 500 mg/l, sulphite, thiosulphate and polythionates may interfere, but currently with no available means of suppression.

**Minimum volume  
of sample.**

500 ml.

**Preservation and  
sample container.**

Field analysis is preferred. If this is impractical, fill glass bottles, completely with sample, avoiding the entrainment of air, acidify with concentrated sulphuric acid to a pH of less than 3 and deliver to laboratory immediately under refrigeration.

**Safety  
considerations.**

Both concentrated sulphuric acid and concentrated alkaline reagent are used in this method. Special care must be exercised in preventing these substances from contacting the skin. Eye protection must be worn to protect against splashes.

## OXYGEN - DISSOLVED

### Winkler Method A

#### 1. Introduction

The Winkler Method (8.2, 8.3, 8.7, 8.8) is based on the titration of free iodine with sodium thiosulphate using starch as an indicator. A white precipitate of manganous hydroxide is formed in the reaction of alkaline potassium iodide with manganous sulphate. Dissolved oxygen oxidizes the precipitate which, when reacted with sulphuric acid, liberates free iodine proportional to the equivalents of dissolved oxygen originally present in the solution.

#### 2. Interferences and Shortcomings

- 2.1 Using the Alsterberg modification of the Winkler method, sodium azide is added with the alkaline reagent to eliminate any nitrite ion present (8.1, 8.4, 8.5).
- 2.2 Ferrous iron interferes when it is preferentially oxidized by dissolved oxygen or by free iodine. Pretreatment of the sample with excess permanganate will convert any ferrous iron to ferric iron. The remaining permanganate must be eliminated by oxalate or it will also interfere (8.4, 8.5).
- 2.3 Ferric iron above 5 mg/l becomes a good oxidizing agent at low pH and will interfere by oxidizing iodide ion to free iodine. The addition of potassium fluoride will effectively suppress this interference (8.2).
- 2.4 Organic compounds may react causing a decrease in DO or a release of iodine from potassium iodide. When acidification releases free iodine, some organic materials can be halogenated. In most cases these reactions are slow, but if the sample is allowed to stand for a period of time before titration, a significant amount of free iodine will be consumed.  
  
It has been experimentally shown that interference from organic matter will cause significant errors when found in excess of 500 mg/l. At present there is no practical method for suppression of organic interference. It is, therefore, imperative that the analytical procedure be performed in the shortest time possible (8.2, 8.4, 8.6).
- 2.5 Interferences by sulphites, thiosulphates, and polythionates are not easily suppressed and can substantially affect the final DO result (8.6).

### 3. Apparatus

- 3.1 Standard BOD bottles or equivalent with well fitted ground glass stoppers or polyseal lined caps.
- 3.2 Balance; analytical (0.1 mg).
- 3.3 Flasks, Erlenmeyer (250 ml).
- 3.4 Titration assembly with burette graduation of 0.05 or 0.10 ml.

### 4. Reagents

- 4.1 Manganese sulphate monohydrate ( $\text{MnSO}_4 \cdot \text{H}_2\text{O}$ ), reagent grade crystals.
- 4.2 Sodium hydroxide ( $\text{NaOH}$ ), reagent grade crystals.
- 4.3 Potassium iodide ( $\text{KI}$ ), reagent grade crystals.
- 4.4 Sodium azide ( $\text{NaN}_3$ ), reagent grade crystals.
- 4.5 Sodium thiosulphate pentahydrate ( $\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$ ), reagent grade crystals.
- 4.6 Starch, soluble reagent grade powder.
- 4.7 Salicylic acid ( $\text{C}_6\text{H}_5(\text{OH}).\text{COOH}$ ), reagent grade crystals.
- 4.8 Sulphuric acid ( $\text{H}_2\text{SO}_4$ ), concentrated reagent grade.
- 4.9 Potassium bi-iodate ( $\text{KH}(\text{IO}_3)_2$ ) reagent grade crystals.

#### 4.10 Manganese Sulphate Solution

Dissolve 364 g manganese sulfate monohydrate in distilled water, filter and dilute to 1 liter.

#### 4.11 Alkali-iodide-azide Reagent

Dissolve 500 g sodium hydroxide and 150 g potassium iodide in 800 ml of distilled water. To this solution add 10 g sodium azide dissolved in 90 ml distilled water. Adjust the final volume to approximately 1 liter.

#### 4.12 Starch Reagent

Prepare a suspension of 6 g soluble starch powder, and 1.25 g salicylic acid in cold distilled water. Add this mixture to about 800 ml of boiling distilled water, with stirring. Allow the solution to boil for a few minutes and adjust the final volume to 1 liter.

#### 4.13 Sodium Thiosulphate Solution (0.0125 N)

Dissolve 6.205 g sodium thiosulphate pentahydrate in freshly boiled and cooled distilled water and dilute to 2 liters in a volumetric flask. Preserve with 1 g sodium hydroxide per liter.

#### 4.14 Potassium Bi-iodate Stock Solution (0.1 N)

Dissolve 3.249 g of potassium bi-iodate in distilled water and dilute to exactly 1 liter.

#### 4.15 Potassium Bi-iodate Standard Solution (0.0125 N)

Dilute 250 ml of potassium bi-iodate stock solution to 2 liters in a volumetric flask (primary standard).

#### 4.16 Standardization of Sodium Thiosulfate

Dissolve approximately 2 g of iodate free potassium iodide in an Erlenmeyer flask containing 100 to 150 ml distilled water. Add 10 ml 1:9 sulphuric acid followed by exactly 10 ml standard bi-iodate solution (0.0125 N). Dilute to approximately 200 ml and titrate with standard thiosulphate solution (0.0125 N). When a pale straw color is reached, add approximately 2 ml of starch solution and complete the titration to the colorless endpoint. Exactly 10 ml of 0.0125 N thiosulphate solution should be required for titration. If necessary readjust the concentration of the thiosulphate solution.

### 5. Procedure

5.1 Fill a BOD bottle to capacity with sample, avoiding excessive aeration with the air.

5.2 Add 2 ml of manganese sulphate solution.

**NOTE:** To minimize mixing with the air, all reagents are added with the tip of the pipette immersed in the sample.

5.3 Add 2 ml alkaline iodide-azide reagent.

**NOTE:** This alkaline solution is extremely corrosive. Wearing of eye and hand protection is necessary.

5.4 Immediately stopper the bottle tightly and shake rapidly by inverting the bottle for 15 to 20 seconds.

**NOTE:** Tap bottle to ensure no entrapment of air bubbles.

5.5 Allow the brown precipitate to settle until at least three-quarters of the top of the solution is clear.

5.6 Acidify with 2 ml concentrated sulphuric acid, being careful not to displace the precipitate.

**NOTE:** Wear eye protection when using concentrated sulphuric acid.

5.7 Stopper tightly. Shake by rapidly inverting the bottle for 15 to 20 seconds. The precipitate will dissolve completely.

5.8 Pipette 100 ml of the clear yellowish-brown solution into a 250 ml Erlenmeyer flask.

5.9 Titrate with standard 0.0125 N sodium thiosulphate solution to a pale straw color. Add about 2 ml starch solution and continue the titration dropwise until the blue solution reaches the colorless endpoint.



5.10 Record the volume of thiosulphate solution used.

**NOTE:** The entire procedure should be carried out as rapidly as possible to minimize the effects of various interferences. Record the thiosulphate volume at the first colourless endpoint. Upon standing the original blue colour will return. This should be ignored.

## 6. Calculation and Reporting

The strength of the titrant is such that 1.0 ml of 0.0125 N sodium thiosulphate solution is equivalent to 1.0 mg/l dissolved oxygen when 100 ml of sample is titrated. The DO in mg/l is numerically equivalent to the volume of sodium thiosulphate titrant in ml.

$$\text{mg/l dissolved oxygen} = \frac{v \times N}{s} \times 8000$$

v = volume of thiosulphate in ml

N = normality of thiosulphate

s = volume of sample in ml

## 7. Precision and Accuracy

Not yet determined.

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**OXYGEN - DISSOLVED**  
**Oxygen Electrode Method B**

**SUMMARY**

<b>Matrix.</b>	This method is used routinely on water, sewage and industrial waste samples.
<b>Substance determined.</b>	Dissolved oxygen.
<b>Interpretation of results.</b>	Results reported as mg/l dissolved oxygen.
<b>Principle of method.</b>	A portion of the dissolved oxygen in a sample diffuses through the gas selective membrane of the oxygen electrode probe into an electrolytic cell where it is reduced at the cathode. The resultant change in current is proportional to the DO concentration in the test sample.
<b>Time required for analysis.</b>	A single analysis requires about 20 seconds.
<b>Range of application.</b>	0.10 - 19.99 mg/l of DO, but values in excess of 10 mg/l are rarely encountered.
<b>Standard deviation.</b>	0.06 mg/l DO.
<b>Accuracy.</b>	Not yet determined.
<b>Detection criteria.</b>	0.10 mg/l DO.
<b>Interferences and shortcomings.</b>	Any gas, which diffuses through the membrane and enters into a redox reaction will interfere; chlorine gas, which is the most frequently encountered chemical of this type, is eliminated by conversion to hypochlorous ion via pH elevation. If the dissolved salt background exceeds 1000 mg/l the rate of diffusion of oxygen is affected, and a correction factor will be required. Prolonged exposure to hydrogen sulphide poisons the lead anode, thus causing slow DO response. The lead anode must be periodically cleaned.
<b>Minimum volume of sample.</b>	60 ml.

**Preservation and  
sample container.**

Field analysis is preferred. However, if this is impossible, the sampling bottle should be completely filled up to avoid entrainment of air. Immediate delivery to the laboratory under refrigeration is essential.

**Safety  
considerations.**

No special precautions are required for the measurements using the oxygen electrode probe. The instrument assembly, however, is standardized against the Winkler method (see Method A), and the appropriate safety measures must be followed while using the Winkler reagents.

## OXYGEN - DISSOLVED

### Oxygen Electrode Method B

#### 1. Introduction

The principle of the oxygen electrode is based on the relationship between the concentration of dissolved oxygen in a sample and the current generated by its reduction under controlled conditions. The electrode probe contains an electrolytic cell separated from test sample by a teflon membrane which is permeable to dissolved oxygen. When the probe is immersed in a sample, a portion of the oxygen in the sample diffuses through the membrane into the electrolytic cell and is reduced at the cathode. The resultant change in current is directly proportional to the oxygen concentration present in the sample, and is read out digitally as mg/l O<sub>2</sub>. A suitable velocity of water across the membrane is maintained by a motorized stirrer and a built-in thermistor compensates for temperature variations.

#### 2. Interferences and Shortcomings

Any gas, which diffuses through the membrane and enters into a redox reaction, will interfere; chlorine is the most commonly known gas of this type and its interference is nullified by raising the pH and converting the gas to hypochlorous ion.

If the dissolved salt content of the sample exceeds 1000 mg/l, the rate of diffusion of oxygen is affected and the DO result will be low. If, however, the composition of this background is constant, a correction factor can be developed.

Prolonged exposure to oily samples will produce a film on the membrane which might retard the diffusion of oxygen. Such films should be wiped off or a fresh membrane should be installed.

Prolonged exposure to samples containing hydrogen sulphide will result in corrosion of the lead anode; periodic cleaning of the electrodes controls this poisoning problem.

#### 3. Apparatus

- 3.1 Oxygen Analyzer, Weston and Stack Inc., Model 350 (Figure 1).
- 3.2 D.O. Probe, Weston and Stack Inc., Model 33.
- 3.3 Flow cell compatible with D.O. Probe Model 33 (Figure 1).
- 3.4 Pre-cut Teflon membranes, 0.5 mm thickness.

#### 4. Reagents

4.1 Sodium sulfite ( $\text{Na}_2\text{SO}_3$ ), reagent grade crystals.

4.2 Potassium iodide (KI), reagent grade crystals.

#### 4.3 Electrolyte Solution

Dissolve 50 g of potassium iodide and 0.1 g of sodium sulfite in 100 ml of distilled water. Store electrolyte in an opaque container.

**NOTE:** The standardization of the oxygen electrode system is based upon comparison with DO results obtained by Method A (Winkler), and thus the reagents of the Winkler procedure are also required.

#### 5. Procedure

5.1 Aerate a sample of distilled water at room temperature for approximately 30 minutes, and determine its DO by the Winkler Method.

5.2 Check the membrane for leakage. Adjust the electronic zero of the Oxygen Analyzer according to the instructions provided in the manual.

5.3 Fill the flow cell with the sample of known DO (predetermined by Winkler procedure) and adjust the Oxygen Analyzer until the same DO reading is displayed. Repeat this calibration every 30 samples to ensure no DO drift is present.

5.4 Analyze samples by filling flow cell and recording the value shown on the digital display as soon as it stabilizes.

#### 6. Calculation and Reporting

The DO as mg/l oxygen is read directly from the Oxygen Analyzer, and is reported to the nearest 0.1 mg/l.

#### 7. Precision and Accuracy

The accuracy of the procedure which depends upon the Winkler Method has not been determined. The standard deviation is 0.06 mg/l DO based on repeated analyses of the same sample.

#### 8. Bibliography

8.1 American Public Health Association, American Water Works Association, and Water Pollution Control Federation (1971). Standard Methods for the Examination of Water and Wastewater. 13th ed. APHA, Washington, D.C.

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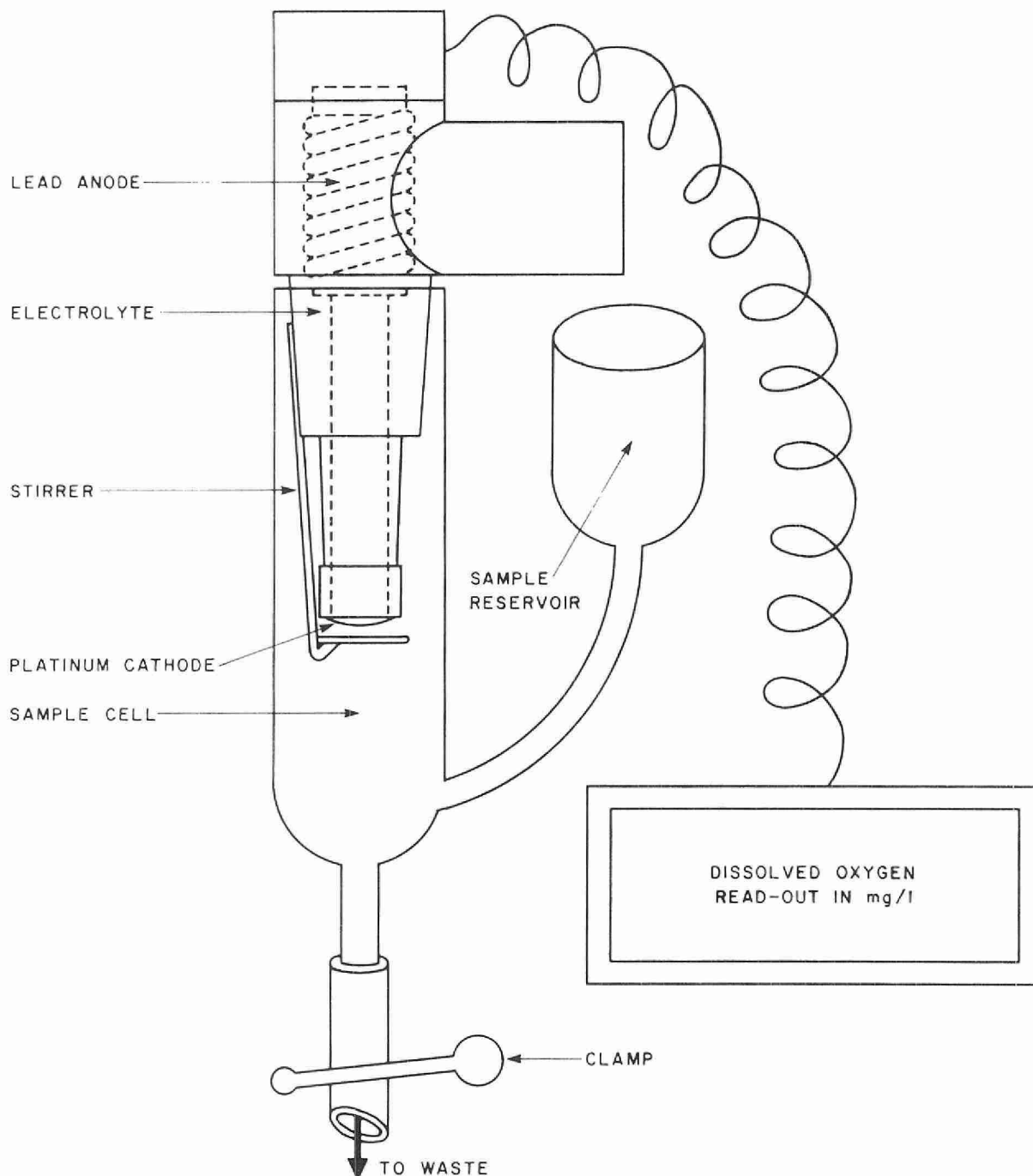


FIGURE 1 — FLOW CELL AND ELECTRODE ASSEMBLY FOR DISSOLVED OXYGEN DETERMINATION METHOD C

## THE DETERMINATION OF PARTICLE SIZE DISTRIBUTION

The solid phase of the soil and sediment is ultimately comprised of structurally diverse organic and inorganic particles which may be crystalline or amorphous and which vary greatly in size and shape. The measurement of particle size is generally restricted to the inorganic component and although these particles are usually clustered as aggregates, their separation is possible by chemical and mechanical means. Many soil textural classification systems are based on particle size measurements, however, the variety of procedures used makes comparisons between classification schemes difficult.

Grain size distribution is one of the most stable soil or sediment characteristics and is little modified by cultivation or other practices. Particle size, however, is a significant controlling factor for such characteristics as porosity, permeability, water holding capacity, leaching potential, exchangeable nutrient supply, cation exchange, weathering behaviour, susceptibility to wind and water transport and the mechanical and geotechnical behaviour of the soil. Owing to its effect on permeability, particle size determination is a useful and sometimes necessary prerequisite to septic tank installation. Furthermore, textural measurements on bottom and suspended sediments from lakes and streams may be used to help determine relationships between water quality, flora and fauna populations and bottom sediment characteristics. The fractionation of soils and sediments into various size classes can also be useful for the correlation of pollutant concentration with texture.

### Sample Handling and Preservation

#### Soil and Sediment

Samples may be collected in glass or plastic containers. No special handling or preservation techniques are required, however, since other tests are often performed on all or part of the sample, the appropriate precautions established for those tests should be observed, with the exception of acid preservation.

### Selection of Method

The method chosen depends on the size range of the particles being examined, the proportion of fines in the sample, the amount of material available for the test and the purposes of the analysis.

All methods involve the separation of larger particles by sieving and the dispersion of smaller particles. Many samples, particularly those with high levels of organic matter, sesquioxides or carbonates require chemical or ultrasonic pretreatment to disaggregate the sample and remove surface coatings around the primary particles (see Method A). However, for some studies, particularly sedimentation studies, it may be desirable to determine particle size without sample pretreatment in order to simulate field conditions.

The sand fraction of a sample is usually determined by a sieve analysis (Method A). The total sand fraction is separated from the silt and clay fraction by wet sieving. It is then



dried, and the size fractions determined by wet or dry sieving using a nest of sieves. When a complete fractionation of the sample is required, the sand is first separated by wet sieving and the remaining silt and clay is dispersed and analyzed by hydrometer analysis (Method B) or pipette analysis (Method C). When using Method B, the sand fraction can be separated by wet sieving before or after the silt and clay determinations.

Most methods currently used for measuring silt and clay fractions are based on the principle of Stoke's Law which relates the settling velocity of spherical particles to their diameter and the difference in specific gravity between particle and dispersing medium. Since particles tend to be plate-like and elongated, rather than spherical, an equivalent spherical diameter is determined. All mineral particles are assumed to have a specific gravity of 2.65; however, if organic matter and surface coatings are not removed, the specific gravity may be significantly different.

Hydrometer analysis (Method B) is the simplest and most rapid method and is satisfactory for use on soils and sediments containing at least 10-20% clay and when a large amount of material is available for the test. The pipet method (Method C) is more laborious and time consuming and requires greater operator care than Method B, but is also more accurate at lower clay contents and requires less sample. The latter is particularly important for suspended sediment analysis.

Sieve size selection and times for hydrometer readings or pipette sampling depend on the particle size classification system requested. The Wentworth Classification and phi units ( $\phi$ ) scale are used primarily in sedimentation studies and differs from the USDA system often used in agricultural work and the AASHO and the Unified Classification used in engineering studies (Figure 1). If a cumulative particle size distribution curve is prepared, it is then possible to interpolate for any classification scheme. The OMOE classification (Figure 1) is used for soil and septic tank drainage tile studies while the Wentworth system is preferred for sedimentation studies since it uses similar particle size ranges to the phi ( $\phi$ ) scheme used by the Canada Centre for Inland Waters.

## PARTICLE SIZE DISTRIBUTION

### Sieve Analysis Method A

#### SUMMARY

<b>Matrix.</b>	This method is used to separate the sand and gravel fractions of soils and sediments.
<b>Substance determined.</b>	Particle size distribution of the sand and gravel fractions of the sample.
<b>Interpretation of results.</b>	The proportions of the various sizes of particles in the sample are represented by frequency ratios within the stated size class or by the relative weights of such classes.
<b>Principle of method.</b>	Soil particles are fractionated into various size classes by passing the sample through a nest of sieves with successively smaller openings from top to bottom.
<b>Time required for analysis.</b>	A single sieve analysis requires about 30 minutes. Pretreatment for organic matter and carbonate removal, however, requires 1 day.
<b>Range of application.</b>	Sieve analysis is applied to particles with diameters of between 12 mm and 0.06 mm. The sieve range may be extended to 75 mm provided suitable sieves are available. Generally, sieves of less than 0.075 mm openings are impractical except under special circumstances.
<b>Standard deviation.</b>	$\pm 5\%$ repeatability for a given size fraction in a given sample.
<b>Accuracy.</b>	The accuracy is dependent on the particle size distribution of the sample.
<b>Detection criteria.</b>	0.0625 mm sieve opening with a mechanical shaker. 0.037 mm for a sonic sifter.
<b>Interferences and shortcomings.</b>	The probability of a particle passing through the sieve in a given length of time depends on the particle and sieve characteristics. Particles cemented together by carbonates or organic matter must first be pretreated to remove these components.

**Minimum volume  
of sample.**

20 - 30 g if hydrometer analysis is to follow, 10 g if pipette analysis is to follow.

**Preservation and  
sample container.**

No preservatives are required. Any wide mouthed glass or plastic container may be used for sample collection.

**Safety  
considerations.**

Normal laboratory safety precautions should be observed.

## PARTICLE SIZE DISTRIBUTION

### Sieve Analysis Method A

#### 1. Introduction

After sample dispersion in a distilled water and Calgon solution, the sand fraction is separated from the silt and clay particles by wet sieving through a No. 10 Tyler sieve. The silt and clay suspension passing through the sieve is retained if a complete particle size distribution is required and then analyzed by Method B or C. Particles remaining on the sieve are dried and then passed through a nest of sieves (by means of a mechanical shaker or sonic sifter). The size fractions are weighed and expressed as a percentage of the original sample weight. These weights may be plotted on semi-log arithmetic graph paper with the particle size as the abscissa, to generate a continuous distribution curve (Figure 2).

#### 2. Interferences and Shortcomings

This outwardly simple procedure has limitations which are not always recognized. The probability of the particle passing through the sieve in a given agitation/washing time depends on the particle and sieve characteristics. A particle whose shape permits passage through the sieve only at a specific orientation or a sieve with unequal openings will limit the opportunities for passage of the particle through the sieve in the allotted time. Particles cemented together by clay, carbonates and/or organic matter may give false particle size readings and therefore proper dispersion of the particles must be achieved. Carbonate, organic matter and iron oxide removal may be achieved with various pretreatments prior to particle size determination. The dispersion of clay is achieved by the addition of Calgon solution to the soil or sediment suspension.

#### 3. Apparatus

- 3.1. Sieve shaker, Tyler Ro-Tap or equivalent, or sonic sifter, Allen-Bradley Model L3 or equivalent.
- 3.2. Sieves, 8 inch bronze wire: 1.5 in (38 mm); 0.75 in (19 mm); 0.25 in (6.3 mm); Tyler Nos. 10, 18, 35, 60, 120 and 230 for the Ro-Tap. The sonic sifter is equipped with Tyler Nos. 20, 44, 110, 170, 235 and 400 sieves.

**NOTE:** These sieve sizes are for use with the Wentworth Classification. Other size fractions may be required for other classification schemes.

- 3.3. Cleaning brushes, camel hair.
- 3.4. Balance, top-loading, 1000 g capacity, 0.01 g sensitivity.
- 3.5. Mortar and pestle, preferably rubber pestle.

- 3.6. Drying oven, capable of  $105^{\circ}\text{C} \pm 2^{\circ}\text{C}$  setting.
- 3.7. Drying trays, dishes or crucibles.
- 3.8. Bottom pans and sieve covers.
- 3.9. Mixer, milkshake type electrically driven, with replaceable stirring paddle or ultrasonic probe, or end over end mixer.
- 3.10. Dispersion cup for mixer.

#### 3.11. Apparatus Required for Carbonate and Organic Matter Removal

- 3.11.1. Centrifuge bottles, 250 ml.
- 3.11.2. Centrifuge, capable of holding 250 ml bottles and of attaining 1500 rpm.
- 3.11.3. Beakers, 1 liter.
- 3.11.4. Hot plate, to heat 1 liter beakers.

### 4. Reagents

- 4.1. Calgon, commercial.

#### 4.2. Calgon Solution (5%)

Dissolve 50 g Calgon in water and dilute to 1000 ml. If Calgon is unavailable use sodium hexametaphosphate and add sodium carbonate to bring the pH to 10 (eg. 35.7 g sodium hexametaphosphate and 7.9 g sodium carbonate) (8.4).

**NOTE:** If dry sieving only is used, or if water dispersion only is performed, these reagents are not required.

#### 4.3. Reagents for Carbonate and Organic Matter Removal

- 4.3.1. Hydrochloric acid ( $\text{HCl}$ ), concentrated, reagent grade.
- 4.3.2. Hydrogen peroxide ( $\text{H}_2\text{O}_2$ ), 30-35%.
- 4.3.3. Silver nitrate ( $\text{AgNO}_3$ ), reagent grade. (Prepare a saturated solution.).
- 4.3.4. Barium chloride ( $\text{BaCl}_2$ ), reagent grade.
- 4.3.5. **Hydrochloric Acid (1 N)**

Dilute 84 ml concentrated hydrochloric acid to 1 liter with distilled water.

### 5. Procedure

If the silt and clay fractions of the sample are to be measured by Methods B or C and the distribution of the mineral particles only is desired, the organic matter and carbonates should be removed by the pretreatments described in 5.1 and 5.2.

(Discretion is advised as carbonate removal on alkaline soils will cause significant loss of sample.)

### 5.1. Carbonate and Soluble Salts Removal

- 5.1.1. Weigh air dried sample (if silt and clay fractions are to be measured weigh 20-30 g for hydrometer analysis and 10 g, if available, for pipette analysis) into 250 ml centrifuge bottle.
- 5.1.2. Add 100 ml water and mix.
- 5.1.3. Add 1 N hydrochloric acid dropwise until a pH of 3.5 - 4.0 is reached and is stable for 10 minutes.
- 5.1.4. Centrifuge at 1500 rpm for 10 minutes. Pour off and discard supernatant.
- 5.1.5. Wash twice with 50 ml distilled water. Shake and centrifuge as in 5.1.4. Discard supernatant.

### 5.2 Organic Matter Removal

- 5.2.1. Add approximately 25 ml water to soil in centrifuge bottle (from step 5.1.5.). Shake and transfer to a 1 liter beaker.
- 5.2.2. Add 5 ml hydrogen peroxide (30-35%) cover and observe for several minutes. If significant frothing occurs, cool under a stream of cold water.
- 5.2.3. Add another 5 ml hydrogen peroxide when frothing subsides.
- 5.2.4. When frothing ceases to occur, heat to 90 °C. Remove cover when reaction has ceased, evaporate excess water (200 ml or less) (do not evaporate to dryness).
- 5.2.5. Repeat steps 5.1.3. and 5.1.4. until most of the organic matter has been destroyed (as judged by the rate of reaction and color of the sample).
- 5.2.6. Occasionally rinse down sides of beaker.
- 5.2.7. Heat for approximately 1 hour after final addition of hydrogen peroxide (do not evaporate to dryness).
- 5.2.8. Transfer the sample back to the centrifuge bottle. Centrifuge and decant.
- 5.2.9. Add approximately 50 ml distilled water, shake and centrifuge.
- 5.2.10. Check the supernatant for salts (a silver nitrate solvent for chlorides; barium chloride for sulphates).
- 5.2.11. Repeat centrifuge washings until salt test is negative.
- 5.2.12. Follow sand separation procedure according to 5.3. and clay and silt determinations according to Methods B or C.

### 5.3. Separation of Sand Fraction

- 5.3.1. If silt and clay are to be determined add 100 ml Calgon solution (5%) to centrifuge bottle and shake.
- 5.3.2. Using a stream of distilled water, transfer suspension to a dispersing cup. Mix suspension for 5 minutes with the milkshake mixer or ultrasonic probe.

- 5.3.3. Alternatively, transfer suspension to 1 liter bottle and mix overnight in end over end shaker.
- 5.3.4. Using a stream of distilled water, wash sample through a fine mesh sieve (230 mesh) (held in a funnel) into a sedimentation cylinder. Silt and clay in the sedimentation cylinder are measured according to Method B or C. Measure sand fraction on 230 mesh sieve according to 5.4 and 5.5. The sieve size used depends upon the soil classification scheme used.

#### 5.4. Dry Sieve Analysis of Sand Fraction

- 5.4.1. Oven dry (105 °C) and weigh fraction on 230 mesh sieve obtained in 5.3.4. If dry sieve analysis only is to be conducted on the sample, weigh out a sample aliquot and disaggregate with mortar and pestle taking care not to break the discrete particles.
- 5.4.2. Assemble a stack of brass sieves for the Ro-Tap from largest (on top) to smallest (on bottom). Use all sieves Nos. 10, 18, 35, 60, 120 and 130 in order to produce a continuous distribution curve or selected sieve sizes depending on fractions required. Ensure that the bottom pan is in place below the finest sieve. For sonic sifter, assemble 5 sieves.
- 5.4.3. Place weighed, oven-dried sample or (for dry sieve analysis only) reweighed disaggregated sample on top sieve in stack and cover with sieve cover.
- 5.4.4. Place stack of sieves in position on sieve shaker.
- 5.4.5. Set timer at 5 minutes for the Ro-Tap and at 10 minutes for the sonic sifter. Normal operating settings for the sonic sifter are: SIFT = 6 - 7 (maximum 8); PULSE = 6 - 7 (maximum 8).
- 5.4.6. Remove stack of sieves and carefully transfer contents of each sieve in succession onto a pre-tared weighing dish. The accumulated weight is recorded after each transfer. Complete the transfer of retained material by carefully brushing the underside of the sieves with a camel hair brush (i.e. to the material in each sieve add the material from the underside of the sieve above it). For a dry sieve analysis only, include contents of bottom pan. If silt and clay determinations are to follow place contents of bottom pan in sedimentation cylinder (5.3.4.).

**NOTE:** Steps 5.4.3. to 5.4.6. may need to be repeated after recombining the separates on samples for which dry sieve analysis only is done and which contain large amounts of fine material, If results are not satisfactory repeat using a smaller sample size. Samples with a high percentage of fines might better be analyzed as in 5.5.

#### 5.5. Wet Sieve Analysis of Sand Fraction

- 5.5.1. For wet sieve analysis only (and determination of fines) weigh an air dried sample aliquot into a 600 ml beaker, add 100 ml calgon solution, stir, and soak overnight. For previously separated sand fraction (5.3.) wash sample from sieve into beaker.
- 5.5.2. Transfer to a dispersion cup using a stream of distilled water.

- 5.5.3. Mix the sample for 5 minutes with mechanical milkshake type mixer or overnight with an end over end shaker.
- 5.5.4. Prepare the Ro-Tap for wet sieving with a stack of sieves as in 5.4.2., start motor and turn on water supply. Alternatively wet sieve manually as follows.  
Prepare a stack of sieves as in 5.4.2. (for wet sieve analysis only no bottom pan is needed). Pour thoroughly mixed sample, or the previously separated sand fraction in the beaker through the stack of sieves ensuring that all particles are washed out of the container.
- 5.5.5. Wash samples through the stack of sieves using a gentle stream of water. Ensure that sieves do not become blocked with fines.
- 5.5.6. Carefully wash each sieve into the one below until sieve effluent is clear.
- 5.5.7. Transfer contents of each sieve into a drying dish by backwashing with a gentle stream of water. Any material which washes through all sieves and collects in bottom pan should be added to sedimentation cylinder and measured with silt and clay determinations.
- 5.5.8. Allow fractions to settle, then carefully decant excess water to reduce drying time. Take care to avoid loss of material during decanting.
- 5.5.9. Place dishes into drying oven and dry at 105°C.
- 5.5.10. Transfer dried material into tared weighing container, transferring largest to smallest fractions in succession. Record accumulated weight after each addition.
- 5.5.11. Determine weight of each fraction separately.

## 6. Calculation and Reporting

$$\% = \frac{(g \times 100)}{w}$$

Where:

g = weight (g) retained on given mesh size  
w = total (original) weight (g) of sample

These percentages can be plotted as a histogram representing the population of each size fraction.

Cumulative percentages may also be calculated and the data recorded in table form, or plotted as a continuous smoothed semi-log curve representing a cumulative size distribution (Fig. 2).

Size fractions for classification schemes for which appropriate sieves are not available can be obtained from the cumulative distribution curve.



## 7. Precision and Accuracy

The average relative error associated with each size fraction is 5% based on replicate analysis of a single sample. The difficulties imposed by samples of varying amounts of clay may cause a larger error on repetitive wet sieving since a separate aliquot must be taken for each retest. Sample pulverization can also affect reproducibility of sieving.

## 8. Bibliography

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## PARTICLE SIZE DISTRIBUTION

### Hydrometer Analysis Method B

#### SUMMARY

<b>Matrix.</b>	This method is used routinely for silt and clay determinations on soil and sediment samples.
<b>Substance determined.</b>	Silt and clay size particle distribution.
<b>Interpretation of results.</b>	The proportion of particles in each size class based on average effective spherical particle diameter. These proportions are represented by the frequency ratios within the stated size classes or by a continuous semi-logarithmic distribution curve. Depending on the type of sample and its preparation, the distribution can be that of the mineral particles or mineral particles with layers of organic matter carbonates or sesquioxides.
<b>Principle of method.</b>	Particles are dispersed in water in a blender and transferred to a sedimentation cylinder and the changes in the density of the suspension with time are measured. Density changes are related to the settling velocity of the particles as determined by Stoke's Law.
<b>Time required for analysis.</b>	A complete analysis requires 24 hours. Approximately 10 samples can be analyzed concurrently. An abbreviated analysis to determine major size classes (silt and clay) requires about 3 hours. If pretreatments and determination of the sand fraction are also done additional time is required.
<b>Range of application.</b>	Typically 62 $\mu\text{m}$ - 2 $\mu\text{m}$ .
<b>Standard deviation.</b>	Not yet determined.
<b>Accuracy.</b>	Dependent upon the nature and actual size distribution of the sample.
<b>Detection criteria.</b>	Detection criteria is a function of sample temperature, density, viscosity of settling medium and the settling time allowed. For a 5% Calgon solution at 20 °C at a particle density of 2.65 g/cm <sup>3</sup> a particle size of 1.4 $\mu\text{m}$ is detectable after a 24 hour settling time at a 15 cm effective hydrometer depth.

**Interferences and shortcomings.**

Material which easily crumbles such as highly weathered rock may increase the percentage of finer material if not handled carefully to prevent disintegration. Light, finely divided organic matter may skew the particle size distribution toward the finer sizes. This material may be removed by sample pretreatment with hydrogen peroxide. The method is based on Stoke's Law of spherical particles falling through a viscous medium and consequently particles which are not spherical and which have a specific gravity other than 2.65 may cause errors. An error of 1 hydrometer scale unit can cause up to 2.5% error in clay percentage.

**Minimum volume of sample.**

20 g. Smaller samples size are acceptable for soils and sediments with greater clay content. Sample size is selected so that the density of the suspension will change from 25 - 10 g/l over the duration of the test.

**Preservation and sample container.**

No preservatives are required. Any wide mouthed glass or plastic container may be used for sample collection.

**Safety considerations.**

Normal laboratory safety precautions should be observed. Eyes, skin and clothing should be protected from direct contact with hydrogen peroxide and hydrochloric acid.

## PARTICLE SIZE DISTRIBUTION

### Hydrometer Analysis Method B

#### 1. Introduction

A portion of sample (which if required, has been pretreated for organic matter and carbonate removal) is dispersed in an electrolyte solution to aid in and maintain the physical separation of charged particles. Samples are dispersed in a sedimentation column, usually after removal of the sand fraction by wet sieving (this is not necessary if the sand fraction is not to be determined). The density of the electrolyte sample suspension is measured at pre-set times using a calibrated standard hydrometer. Calculations based on Stoke's Law of spherical particles falling through a viscous medium, allow for the determination of particle sizes at a given time and solution depth. Density measurements at these times are used to determine the concentration of particles remaining in suspension at a given depth.

#### 2. Interferences and Shortcomings

This method for particle size analysis requires particle dispersion in an aqueous solution. In many cases, shaking the soil in a dilute alkaline solution of sodium metaphosphate is sufficient for dispersion of all except the finest colloidal aggregates. However, soils and sediments containing considerable amounts of gypsum, organic matter or readily soluble salts may not disperse adequately unless these components are first removed. Removal can be accomplished by pretreating the soil with peroxide to destroy organic matter and washing with water to remove gypsum. In some cases, treatment with dilute hydrochloric acid is required for the removal of carbonates and iron or manganese coatings around mineral grains.

The assumption that the particles behave according to Stoke's Law for spherical particles may not always hold true since the particles are often not spherical and do not always have a specific gravity of 2.65. The largest analytical error in the method is associated with the reading of the hydrometer. An error of 1 hydrometer scale reading can cause up to 2.5% error in clay percentage. This is significant, particularly at low clay contents.

Other minor sources of error include a change in settling velocity due to friction on the sides of the cylinder, the tendency for very small clay particles to remain on the surface and minor turbulence caused by lowering the hydrometer into the sedimentation cylinder.

#### 3. Apparatus

- 3.1. Hydrometer, standard, ASTM No. 152H with Bouycoucos scale in g/l.
- 3.2. Graduated cylinder, 1 liter with 1000 ml mark,  $36 \pm 2$  cm from the bottom on the inside of the cylinder.

- 3.3. Brass or plastic plunger, with 5.4 cm diameter plate attached to 50 cm long rod.
- 3.4. Water bath, 58 x 48 x 32 cm, capacity to hold at least 10, 1 liter cylinders.
- 3.5. Balance, accurate to 0.1 g.
- 3.6. Also 3.9. - 3.11. Method A, for particle pretreatment and dispersion.

#### 4. Reagents

As for Method A.

#### 5. Procedure

- 5.1. Weigh a 20-30 g sample of air dried sediment. For organic matter and carbonate removal, pretreat according to 5.1. and 5.2. Method A. The sample is then dispersed and the sand fraction removed according to 5.3. Method A.

**NOTE:** If organic matter and carbonates are not removed, eliminate steps 5.1 and 5.2 Method A. If the sand fraction is to be separated after silt and clay determination, only 5.3.1. and 5.3.2. Method A need be completed.

- 5.2. Once in the sedimentation cylinder, allow to stand overnight in a water bath at room temperature. The following morning check for completeness of dispersion (fine clay should still be in suspension). Record the temperature of the water bath.

##### 5.3. Calibration of Hydrometer

- 5.3.1. Add 100 ml of 5% Calgon solution to a cylinder and make to volume with distilled water. Allow this cylinder to also stand overnight at room temperature in the water bath.

- 5.3.2. Lower the hydrometer into the cylinder and determine the scale reading ( $R_L$ ) at the upper edge of the meniscus surrounding the stem of the hydrometer.

**NOTE:** If a constant temperature room is not available measure  $R_L$  at the temperature likely to occur.

- 5.4. Insert plunger and move up and down to mix contents thoroughly (hold cylinder firmly in place when plunger is pulled upward). To avoid spilling contents and creating undue turbulence move plunger cautiously near the top of the suspension taking care not to lift plunger out of the suspension. Use strong upward strokes near bottom to lift any particles lodged there. To dislodge sediment remaining in lower corners, incline rod slightly and rotate. Finish with 2 or 3 smooth strokes and remove plunger, tipping it slightly to remove adhering drops. Begin recording time immediately. If surface is covered with foam, add a drop of amyl alcohol.

Alternatively, invert cylinders to mix, closing top of cylinder. Place cylinder in bath.

- 5.5. For a complete particle size distribution, carefully lower hydrometer into suspension and read scale at top of meniscus. Without removing hydrometer, read again at the end of 1 minute. Carefully remove hydrometer (after this and each subsequent reading). Record the reading  $R_0$  at each time.
- 5.6. Without remixing suspension between measurements, carefully lower hydrometer into suspension about 10 seconds prior to each measurement. Take measurements at 3, 10, 30, 90, 270 and 720 minutes or at other times determined by the size class desired. If wet or dry sieve analysis of the sand fraction is to be conducted, readings at the earlier time intervals can be omitted.
- 5.7. If the sand fraction is to be sieved after silt and clay determination, pour sample through a 230 mesh sieve and analyze as in 5.4. or 5.5. Method A.

## 6. Calculation and Reporting

### 6.1. Calculation Procedure

- 6.1.1. Record hydrometer readings ( $R_0$ ) and actual elapsed time on form. For each reading calculate summation percentage.

$$P\% = 100 C/C_0$$

Where:

- $C_0$  = original oven-dried sample weight used for test (g/l)  
 $C$  = hydrometer reading minus density correction for the suspension  $C = R_0 - R_L$ . ( $R_0$  and  $R_L$  were previously defined in 5.3 and 5.5).

- 6.1.2. Calculate corresponding particle size or "diameters" from the equation:

$$D \text{ (micrometers)} = \theta/t^{1/2}$$

Where:

- $D$  = particle diameter  
 $t$  = sedimentation time (minutes)  
 $\theta$  = sedimentation parameter obtained from Table 1, determined by the observed value  $R_0$

Programs can be written for a programmable calculator or computer.

- 6.1.3. For analysis conducted at a temperature other than 30°C use the viscosity of water to calculate a corrected sedimentation parameter.

$$\theta_C = \theta(V_t/V_{30})^{1/2}$$

Where:

- $\theta$  = sedimentation parameter  
 $\theta_C$  = corrected sedimentation diameter  
 $V_t$  = viscosity of water at the recorded temperature (Table 3)  
 $V_{30}$  = viscosity of water at 30°C

The correction for temperature can be included in the calculation of particle diameter as follows:

$$D(\mu\text{m}) = \frac{\theta(V_t/V_{30})^{1/2}}{t^{1/2}}$$

6.1.4. When the particle density is other than 2.65 g/cm<sup>3</sup> a density correction is made as follows (see Table 2):

$$d_c = d \times b$$

b = correction factor

d = density

Table 1 Values of  $\theta$  for Corresponding Values of  $R_0$

R	$\theta$	R	$\theta$	R	$\theta$
-5	50.4				
-4	50.1				
-3	49.9				
-2	49.6				
-1	49.4				
0	49.2				
1	48.9				
2	48.7				
3	48.4				
4	48.2				
5	47.9				
6	47.7				
7	47.4				
8	47.2				
9	47.0				
10	46.7				
		11	46.4	26	42.2
		12	46.2	27	41.9
		13	45.9	28	41.6
		14	45.6	29	41.3
		15	45.3	30	41.0
		16	45.0	31	40.7
		17	44.8	32	40.4
		18	44.5	33	40.1
		19	44.2	34	39.8
		20	43.9	35	39.5
		21	43.7	36	39.2
		22	43.4	37	38.9
		23	43.1	38	38.6
		24	42.8	39	38.3
		25	42.5	40	38.0

**Table 2 Values of Correction Factor, b, for Different Specific Gravities of Soil Particles**

Specific Gravity	Correction Factor b
2.95	0.94
2.90	0.95
2.85	0.96
2.80	0.97
2.75	0.98
2.70	0.99
2.65	1.00
2.60	1.01
2.55	1.02
2.50	1.03
2.45	1.05

**Table 3 Viscosity of Water**

$^{\circ}\text{C}$	Viscosity	$^{\circ}\text{C}$	Viscosity
15	1.139	26	0.8705
16	1.109	27	0.8513
17	1.081	28	0.8327
18	1.053	29	0.8148
19	1.027	30	0.7975
20	1.002	31	0.7808
21	0.9779	32	0.7647
22	0.9548	33	0.7491
23	0.9325	34	0.7340
24	0.9111	35	0.7194
25	0.8904		

- 6.2.5. Plot summation percentages against corrected particle diameter on semi-log paper as in Figure 2.
- 6.2.6. A population histogram may be prepared by plotting the percentage of each size fraction against the size fraction.
- 6.2.7. A cumulative particle distribution curve can be plotted using the results of both the hydrometer and sieve analysis.

## 7. Precision and Accuracy

The average relative error associated with each size fraction is 5% based upon repeated analysis of a single sample. Variations in the percentage of fines and coarse material have a large effect on the reproducibility of the test.



## 8. Bibliography

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## PARTICLE SIZE DISTRIBUTION

### Pipet Analysis Method C

#### SUMMARY

<b>Matrix.</b>	This method is used for silt and clay determination on soil and sediment samples.
<b>Substance determined.</b>	Particle size distribution of silt and clay size material.
<b>Interpretation of results.</b>	The proportion of particles in each size class based on average effective spherical particle diameter. These proportions are represented by the frequency ratios within the stated size classes or by a continuous semi-logarithmic distribution curve. Depending on the type of sample and its preparation, the distribution can be that of the mineral particles or mineral particles with layers of organic matter carbonates or sesquioxides.
<b>Principle of method.</b>	Particles are dispersed as for Method B. Dispersed particles of the same specific gravity settle at a velocity dependent on their equivalent spherical diameters (Stoke's Law). Aliquots of the suspension are removed by pipette from a constant depth at various settling times calculated to represent particles of specified average diameters.
<b>Time required for analysis.</b>	10 samples can be analyzed in 24 hours.
<b>Range of application.</b>	2 $\mu\text{m}$ - 62.5 $\mu\text{m}$ .
<b>Standard deviation.</b>	0.3 - 0.7 based on quadruplicate analysis (8.4).
<b>Accuracy.</b>	Not yet determined.
<b>Detection criteria.</b>	Dependent on particle size distribution and accuracy of weighing.
<b>Interferences and shortcomings.</b>	Coatings of organic matter, iron and manganese oxides and carbonates may alter the specific gravity of the particle and affect its settling velocity. This results in erroneous measurements of the effective spherical diameter of the mineral particles unless pre-treatment is used.

**Minimum volume  
of sample.**

1 g dispersed in 500 ml water.

**Preservation and  
sample container.**

No preservatives are required. Any wide mouthed container can be used. Stabilize material by drying at a maximum temperature of 60°C.

**Safety  
considerations.**

Normal laboratory safety precautions.

## PARTICLE SIZE DISTRIBUTION

### Pipet Analysis Method C

#### 1. Introduction

This method is similar to the hydrometer analysis Method B in that it is based on the settling velocity of spherical particles through the application of Stoke's Law. Particles of a specific average size are collected by withdrawing a sample of the suspension at a predetermined time. Samples are then dried and the weight of suspended material determined. Separation of larger particles (sand size or greater) is achieved by sieving, pretreatment and dispersion steps prior to silt and clay determination as described in Method A 5.1, 5.2 and 5.3. If the particle size distribution of the sand fraction is to be determined the entire procedure in Method A should also be used.

#### 2. Interferences and Shortcomings

This method for particle size analysis requires particle dispersion in an aqueous solution. In many cases, shaking the soil in a dilute alkaline solution of sodium hexametaphosphate and sodium carbonate is sufficient for dispersion of all except the finest colloidal aggregates. However, soils and sediments containing considerable amounts of gypsum, organic matter or readily soluble salts may not disperse adequately unless these components are first removed. Removal can be accomplished by pretreating the soil with peroxide to destroy organic matter and washing with water to remove gypsum. In some cases treatment with dilute hydrochloric acid is required for the removal of carbonates and iron or manganese coatings around mineral grains.

The assumption that the particles behave according to Stoke's Law for spherical particles may not always hold true since the particles are often not spherical and do not always have a specific gravity of 2.65.

Other minor sources of error include a change in settling velocity due to friction on the sides of the cylinder, the tendency for very small clay particles to remain on the surface and minor turbulence caused by lowering the pipet into the sedimentation cylinder.

#### 3. Apparatus

- 3.1. Suction filtering apparatus with two-way stopcock and capillary sized to permit filling pipette in 30 seconds.
- 3.2. Pipette, 20 ml, with three-way stopcock and 200 mm stem, 90 mm long, 20 mm OD barrel; tip opening 2.5 mm I.D. tapered and ground flat.
- 3.3. Pipette holder with sliding clamp for lowering and positioning pipette at 10 cm below upper mark.

- 3.4. 500 ml or 1 liter cylinders.
- 3.5. Sampling dishes, aluminum or fused silica crucibles, 35 ml.
- 3.6. Beaker, 50 ml.
- 3.7. Drying oven, capable of maintaining 105<sup>0</sup> C.
- 3.8. Desiccator.
- 3.9. Balance, accurate to 0.0001 g. Preferably computer interfaced.
- 3.10. Also 3.2 - 3.5 Method B.
- 3.11. 3.9 - 3.11 Method A, for particle pretreatment and dispersion.

#### 4. Reagents

As for Method A.

#### 5. Procedure

- 5.1. Weigh out 10 g sample. For organic matter and carbonate removal, follow steps 5.1. and 5.2. Method A. Disperse sample and remove sand fraction according to 5.3. Method A.
- 5.2. The sample, which has been washed through the 230 mesh sieve (sieve size depends on soil classification scheme used) is collected in a sedimentation cylinder and diluted with water to 1 liter.
- 5.3. Note temperature of water in the control cylinder. Select appropriate sampling time for various size fractions at specified sampling depths. Some sampling times at given depths are given in Table 7, however, depending upon the classification system and particle sizes required, other times may be computed.
- 5.4. Stir suspension for 1 minute using brass plunger as described in 5.3. Method B. Alternatively invert cylinder to mix, sealing cylinder. Begin recording time immediately after mixing.

**Table 4 Sampling Times for Pipet Analysis**

Temp °C	10 cm 50 µm	10 cm 20 µm		10 cm 5 µm		5 cm 2 µm		Sampling depth particle diameter time
	sec	min	sec	hr	min	hr	min	
20 <sup>0</sup>	44	4	40	1	5	3	50	
25 <sup>0</sup>	40	4	5	1	6	3	30	
30 <sup>0</sup>	34	2	40	0	58	3	3	
35 <sup>0</sup>	30	2	20	0	51	2	28	
(8.3)								

**NOTE:** The above sampling times assume a specific gravity for the soil particles of 2.65.

- 5.5. Ensure that the stopcock on the sampling pipet is closed.
- 5.6. Adjust the position of the 20 ml pipette so that the tip rests on the surface of the suspension in the cylinder.
- 5.7. Note the required sampling time and depth and lower pipette into the suspension approximately 20 seconds before actual time of sampling.
- 5.8. Open stopcock on pipette at the correct sampling time and begin sucking aliquot steadily. When pipette is full close stopcock and discharge any excess liquid into a 50 ml beaker.

**NOTE:** Take extreme care not to discharge aliquot back into the cylinder at this point. The stopcock on the pipette is a 3-way type and it is necessary that the sampler be familiar with its operation before sampling.

- 5.9. Discharge the 20 ml aliquot into an aluminum sampling dish or crucible which has been previously labelled and tared to within 0.0001 g.
- 5.10. Dry dish and sample overnight in oven at 105°C. Record weight of oven dried residue after cooling in a desiccator.
- 5.11. Repeat above procedure for all sample sizes.

**NOTE:** The weight of dispersant in a 20 ml aliquot of sample must also be determined. To do this mix 100 ml Calgon solution in 1 liter of distilled water. Place 20 ml of this solution in a tared weighing dish and evaporate to dryness overnight, weigh. The weight of the dispersant is subtracted from the sample weight as in 6.

- 5.12. If organic matter has not been removed by hydrogen peroxide prior to analysis, the organic matter content may be determined by ignition at 475°C (using silica crucibles). However, appreciable adjustment in the calculation may be required due to significant changes in the specific gravity of the sample.

## 6. Calculation and Reporting

The percentages of the particle size fractions are calculated on an organic matter-free and carbonate-free basis. The weight of sand retained on the sieve represents the total sand content of the sample (Method A). The 20 ml aliquots of the silt (<62.5 µm) and clay suspension (<2 µm) represent 1/50th of the mineral sample in suspension. To obtain total weights of the fractions apply a factor of 50 to each sample. Also correct for the weight of calgon in each aliquot.

An example of the results of a partial analysis (2 sampling times) are as follows:

1)	Weight of sand fraction (Method A)	4.7050
2)	Weight of silt + clay + calgon in 20 ml	0.6041
3)	Weight of clay + calgon in 20 ml	0.5766
4)	Weight of calgon in 20 ml	0.5000
5)	Weight of silt in 20 ml (2-3)	0.0275
6)	Weight of clay in 20 ml (3-4)	0.0766
7)	Total weight of silt in sample (50 x 5)	1.3750
8)	Total weight of clay in sample (50 x 6)	<u>3.8300</u>
		9.9100 g TOTAL

% sand	47.47
% silt	13.88
% clay	<u>38.65</u>

100.00% TOTAL

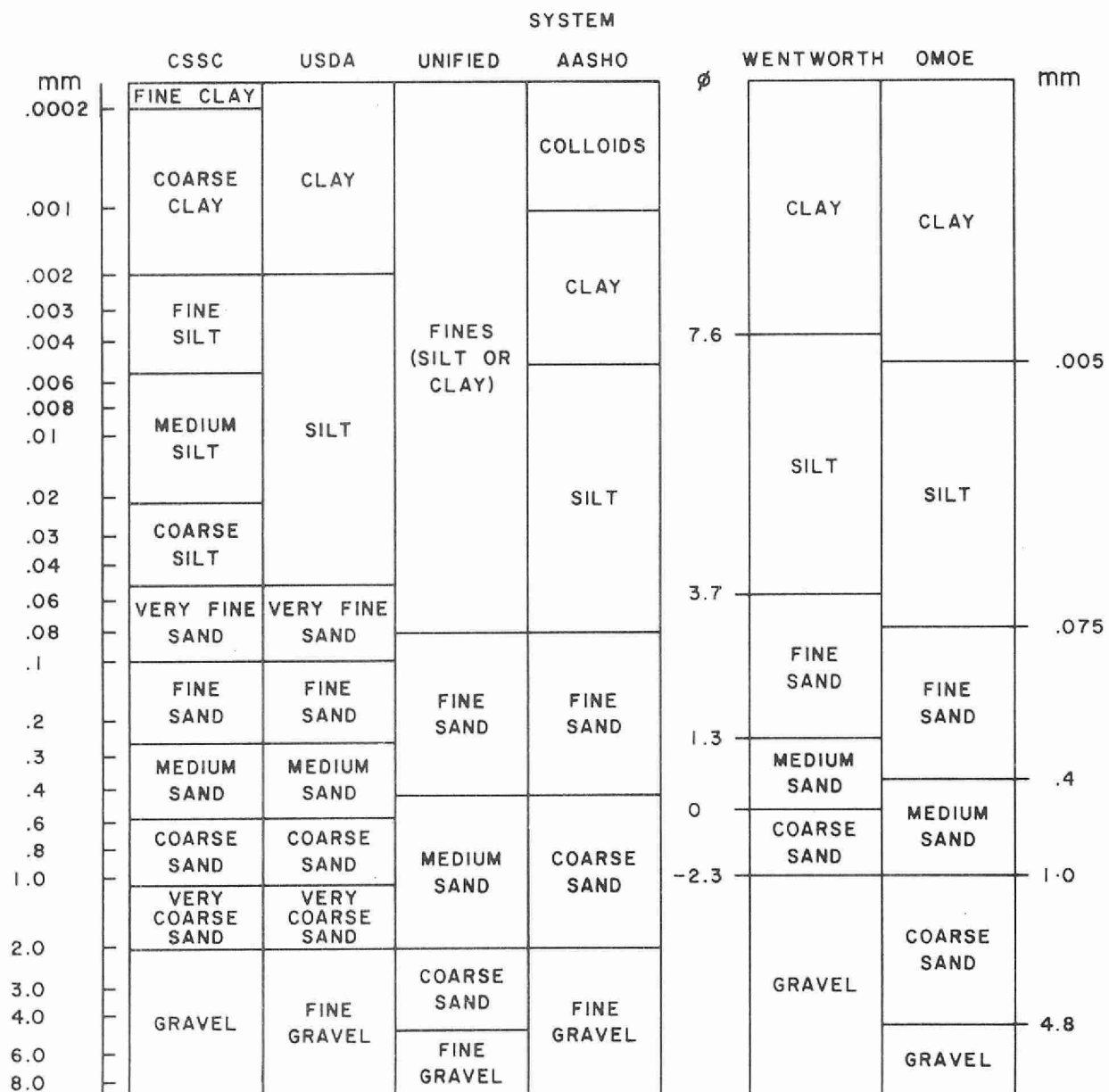
**NOTE:** With a computer interfaced electronic balance weights can be stored in a cassette and a mini-computer system (eg. HP 9830) can be used to facilitate calculations.

## 7. Precision and Accuracy

Based on quadruplicate analysis the standard deviation of this technique was found to be between 0.3 and 0.7 (8.5).

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#### LEGEND

CCSC - CANADIAN SOIL SURVEY COMMITTEE

USDA - UNITED STATES DEPARTMENT OF AGRICULTURE

AASHO - AMERICAN ASSOCIATION OF STATE HIGHWAY OFFICIALS

OMOE - ONTARIO MINISTRY OF THE ENVIRONMENT

(Adapted from McKeague, 1978)

FIGURE 1 — PARTICLE SIZE LIMITS OF SIX PARTICLE SIZE CLASSIFICATION SYSTEMS



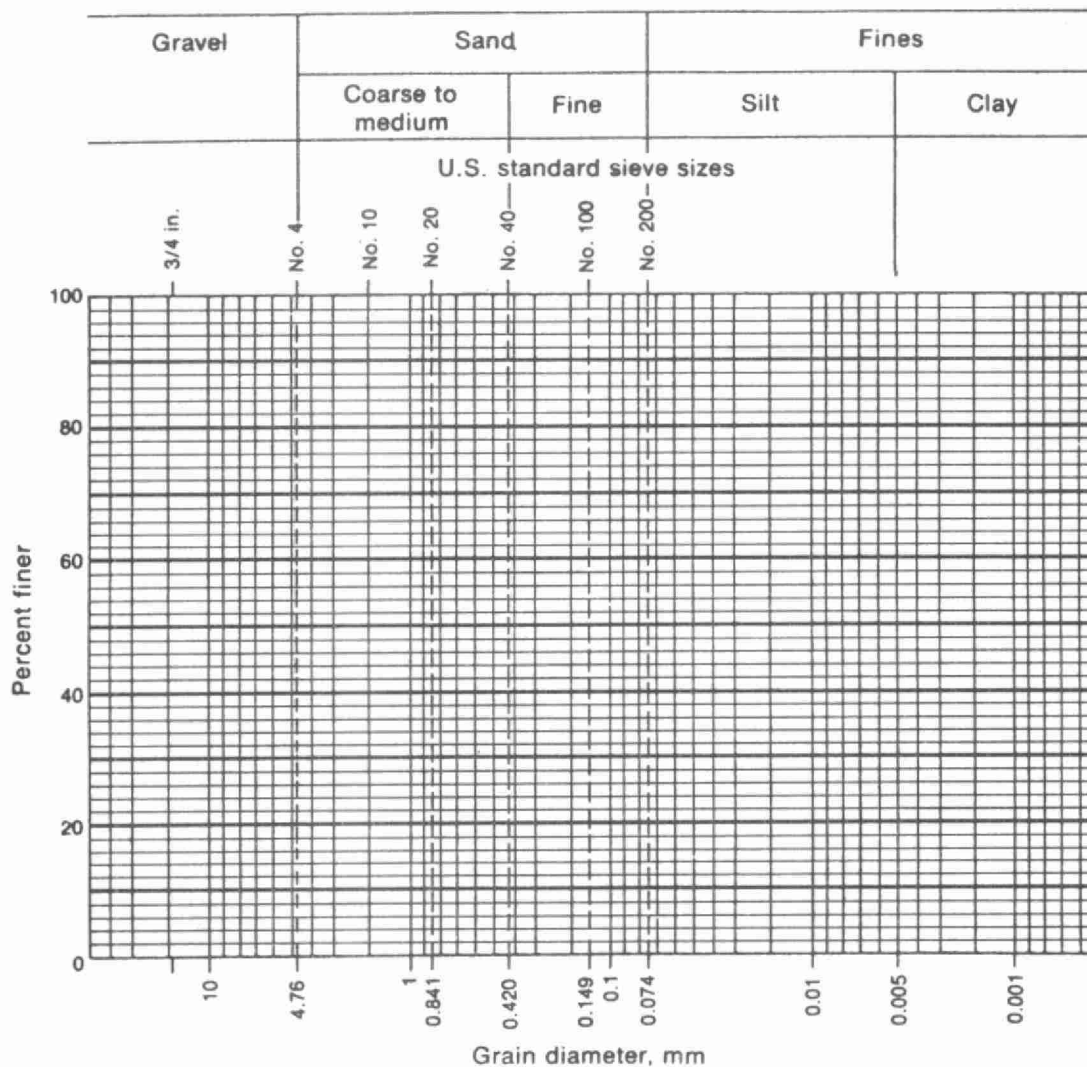
# GRAIN SIZE DISTRIBUTION

Project \_\_\_\_\_ Job. No. \_\_\_\_\_

Location of Project \_\_\_\_\_ Boring No. \_\_\_\_\_ Sample No. \_\_\_\_\_

Description of Soil \_\_\_\_\_ Depth of Sample \_\_\_\_\_

Tested By. \_\_\_\_\_ Date of Testing \_\_\_\_\_



Visual soil description \_\_\_\_\_

Soil classification:

\_\_\_\_\_ System \_\_\_\_\_

FIGURE 2 — STANDARD FORM FOR PLOTTING CUMULATIVE GRAIN SIZE DISTRIBUTION CURVES

## THE DETERMINATION OF PETROLEUM HYDROCARBONS

Approximately 90% (by weight) of the organic chemicals the world uses today come from petroleum and natural gas. In 1979 Canada consumed 666 million bbl of crude oil, or 1,850,000 bbl/day. A considerable portion of this vast quantity of crude oil is transported by pipeline to Ontario where it is refined into various petroleum distillates such as internal combustion engine fuels, heating oils, lubricants, and petrochemical feedstock. A summary of distillate products and their uses is presented in Figure 1.

The potential of petroleum hydrocarbons as environmental pollutants is readily predictable when it is considered that at any one time millions of gallons of petroleum products are being used, transported and stored throughout the province. Accidental spillage of any fraction may cause a variety of problems including the pollution of water supplies, soils, sediments and vegetation. Spill during transport, small but frequent spills resulting from careless handling at gasoline stations, improperly installed plumbing in storage facilities and storage tank leakage caused by corrosion are major contributors to this problem.

Spilled petroleum distillate may contaminate nearby surface waters and penetrate into the ground water table where it rapidly distributes over a large area, contaminating domestic wells. Taste and odor problems develop and the water becomes unacceptable for drinking. The majority of samples received for petroleum hydrocarbon analysis are from rural areas where drinking water availability is dependent on dug or drilled wells.

### Sample Handling and Preservation

#### Water

The sample should be submitted in a clear 1 liter glass bottle with either an aluminum foil or Teflon cap liner. If there is a delay in shipping samples to the laboratory, refrigeration is necessary to minimize volatilization of components in the sample.

Since gasolines and fuel oils float on water, surface samples should be obtained whenever possible. Where a tap sample from a closed system is the only alternative, the tap should not be opened for several hours prior to sampling; this allows a build-up of the pollutant at the valve. The morning is a good time to take tap samples after an all night accumulation of the contaminant in the pipe. If a specific gasoline or fuel oil is suspected of causing the contamination, a sample of it should be submitted for comparison with the contaminant in the sample. It is important to establish that pollution exists, but it is more important to establish the source of the pollutant.

#### Soils and Sediments

Soil and sediment samples should be collected in glass pomade jars. No preservative is necessary but samples should be transported to the laboratory for analysis as soon as possible.

### **Selection of Method**

The gas chromatographic method is used for the determination of gasolines and fuel oils in liquid and soil samples.

## PETROLEUM HYDROCARBONS

### Gas Chromatographic Method

#### SUMMARY

<b>Matrix.</b>	This method is routinely used for petroleum hydrocarbon determination on water, soil and sediment samples.
<b>Substance determined.</b>	Gasolines and distillate fuel oils.
<b>Interpretation of results.</b>	Weathering of the sample may alter that part of the chromatogram which is used for quantitative purposes. Consequently, although the reported results are considered to be semi-quantitative only, they are none-the-less indicative of the magnitude of the pollution present (liquid samples only). The qualitative results are based on direct comparison with known standards. The source of the contaminant can often be identified by comparing the chromatogram of the extracted pollutant with those of samples collected from storage tanks in the spill area.
<b>Principle of method.</b>	The contaminant is extracted from the sample with an organic solvent and an aliquot of the extract is injected into a gas chromatograph. The resulting chromatogram is assessed qualitatively and semi-quantitatively for liquid samples, and qualitatively for soil samples.
<b>Range of application.</b>	Not applicable.
<b>Time required for analysis.</b>	5 - 10 samples per day.
<b>Standard deviation.</b>	Data not available.
<b>Accuracy.</b>	Data not available.
<b>Detection Limit.</b>	>0.1 mg/l gasoline and 0.5 mg/l fuel oil (semi-quantitative) for liquid samples only.
<b>Interferences and shortcomings.</b>	No single standard exists for gasolines and fuel oils, since major component ratios can vary between refineries and even between batches. Gasolines and fuel oils are susceptible to rapid deterioration due to volatilization; oxidation and bacterial action can alter their compositions and characteristics. Consequently, the chromatograms produced may show distortion, and some of the lower boil-

ing components may be missing. Gasolines and fuel oils are partially absorbed onto the walls and cap liners of their containers. Thus, the containers, as well as their samples, must be solvent-extracted. Due to the penetrating odor of petroleum products, the human nose can detect their presence at levels which are too low for detection by gas chromatography.

**Minimum volume  
of sample.**

1 liter bottle completely filled (no head space) and 200 g soil.

**Preservation and  
sample container.**

1 liter glass bottles with aluminum foil or Teflon lined caps for water and pomade jars for soils.

**Safety  
considerations.**

Vapors of n-hexane and the sample itself containing large amounts of gasoline and/or distillate fuel oils are highly flammable.

## PETROLEUM HYDROCARBONS

### Gas Chromatographic Method A

#### 1. Introduction

Water samples are extracted with n-hexane, and soil and sediment samples are treated with dried magnesium sulphate and either packed into a chromatographic column and eluted with n-hexane, sonically extracted with n-hexane and concentrated or extracted using a Soxhlet apparatus. An aliquot of extract or eluent is injected into a gas chromatograph and the resulting chromatograph interpreted.

#### 2. Interferences and Shortcomings

During the extraction of liquid samples, emulsions may form which could result in a loss of the components of interest. During sample preparation of soil and sediment samples, the eluent may have to be reduced to concentrate the sample. This step could result in the loss of some of the more volatile components.

#### 3. Apparatus

- 3.1. Separatory funnel, 2,000 ml with Teflon stopcock.
- 3.2. Syringe or pipette, 1 ml.
- 3.3. Centrifuge tube, 15 ml.
- 3.4. Syringe, Hamilton #801, 10  $\mu$ l or similar.
- 3.5. Sonic bath (soil and sediment samples).
- 3.6. 4 oz pomade jar with screw cap (soil and sediment samples).
- 3.7. All glass Soxhlet extractor (soil and sediment samples).
- 3.8. Glass chromatographic column, 34 x 1.5 cm (soil and sediment samples).
- 3.9. Varian Aerograph model 1400 and Hewlett Packard model 5720A gas chromatographs. For gasolines and fuel oil distillates use a 6 ft long stainless steel column with 1/8" O.D., 80 - 100 mesh Chromosorb W solid support, flame ionization type detector. For characterization of the environmental weathering on gasolines use an 8 ft stainless steel column with 1/8" O.D., a 100 - 120 mesh Supelcoport solid support and a flame ionization type detector.
- 3.10. Dual pen recorder, Varian model 9176.
- 3.11. Shimadzu "Chromatopac EI-A" electronic integrator and printer.

#### **4. Reagents**

- 4.1. n-hexane ( $C_6H_{14}$ ), distilled in glass.
- 4.2. Magnesium sulphate ( $MgSO_4$ ), oven dried (soil and sediment samples only).
- 4.3. Potassium chloride (KCl), reagent grade crystals.
- 4.4. Florisil, for chromatographic analysis.
- 4.5. Aluminum Oxide ( $Al_2O_3$ ) (alumina), acidic (soil and sediment samples only).

#### **4.6. Standards**

Dilute known amounts of gasoline or fuel oil with n-hexane to provide a series of working standards.

#### **5. Procedure**

##### **5.1. Sample Extraction - Water**

- 5.1.1. Add 1 ml n-hexane and 100 g potassium chloride to approximately 800 - 900 ml sample in bottle. Tightly cap bottle and shake vigorously for 5 minutes.
- 5.1.2. Allow hexane to separate. If necessary, centrifuge to further separate hexane from water. Analyze extract according to 5.5.

##### **5.2. Sample Extraction - Soil and Sediment Alternative A**

- 5.2.1. Place a 10 - 30 g sample in a pomade jar and add 25 - 50 g magnesium sulphate. Cap jar and shake until soil is dry.
- 5.2.2. Pack contents of jar into chromatographic column containing 2 g Florisil and 2 g acidic alumina.
- 5.2.3. Elute contents of column with n-hexane. Retain the first 5 ml of eluent. Analyze as in 5.5.

##### **5.3. Sample Extraction - Soil and Sediment Alternative B**

- 5.3.1. Place a 30 g sample and 50 g magnesium sulphate in a pomade jar. Cap and shake until soil is dry.
- 5.3.2. Add sufficient n-hexane to cover sediment.
- 5.3.3. Extract using Sonic Bath, for 15 minutes.
- 5.3.4. Separate n-hexane solution, filter and concentrate under reduced pressure to a final volume of 5 ml using a rotary evaporator. If further concentration is necessary reduce to 1 ml using a stream of dry nitrogen or helium. Analyze as in 5.5.

#### 5.4. Sample Extraction - Soil and Sediment Alternative C

- 5.4.1. Combine 10 g soil and 10 g magnesium sulphate in pomade jar and shake until soil is dry.
- 5.4.2. Fill suitable size cellulose extraction thimble and use Soxhlet apparatus for extraction with n-hexane. Use at least 40 extraction cycles.
- 5.4.3. The resulting extract will be concentrated under reduced pressure to 5 ml. If concentration to 1 ml is necessary use stream of dry nitrogen or helium. Analyze as in 5.5

#### 5.5. Gas Chromatographic Analysis

##### 5.5.1. Operating Conditions (Gasoline and Fuel Oils)

Stationary phase: 10% SP 2100  
Temperature: injector 250°C  
                  detector 350°C  
Column temperature (gasoline):  
                          initial 80°C  
                          final 180°C  
                          rate 12°C/min.  
Column temperature (distillate fuel oil):  
                          initial 120°C  
                          final 250°C  
                          rate 12°C/min.  
Gas flow rate: carrier gas (N<sub>2</sub>) 35 ml/min.  
                  hydrogen (H<sub>2</sub>) 35 ml/min.  
                  air 250 ± 10 ml/min.  
Chart speed: 1 cm/min.

##### 5.5.2. Operating Conditions (for Environmental Change Characterization of Gasolines)

Stationary phase: 5% SP 1200  
                      1.75% Bentone 34  
Temperature: injector 250°C  
                  detector 300°C  
Column temperature: initial: 60°C  
                          final: 170°C  
                          rate: 12°C/min.

- 5.5.3. Inject 5 µl of extract or eluent into the gas chromatograph. The resulting chromatogram is visually compared to those produced by known amounts of gasoline and fuel oil in n-hexane used for standardization. Examples of resulting chromatograms are seen in Figure 2.

**NOTE:** If injection contains insufficient material for identification from sediments reduce 5 ml eluent to 1 ml by a stream of dry nitrogen. Inject 5 µl of reduced eluent into gas chromatograph.



## 6. Calculation and Reporting

Each run is calibrated with known concentrations of gasoline and distillate fuel oil. The total area integrated by a selected retention time window is compared with that obtained from injections of unknown concentrations. Any attenuation changes are taken into account.

Absolute values obtained by this analysis for gasoline and fuel oils are acceptable only on a semi-quantitative basis due to lack of universal standards and the uncontrollable weathering effect caused by environmental changes. If procedure is correctly followed, however, values obtained are acceptable for monitoring purposes to evaluate the environmental fate of contaminants. Meaningful evaluation of numerical values is only possible if analyst has strong environmental organic chemistry hydrogeology and analytical chemistry background.

## 7. Precision and Accuracy

Data not available. Test is semi-quantitative.

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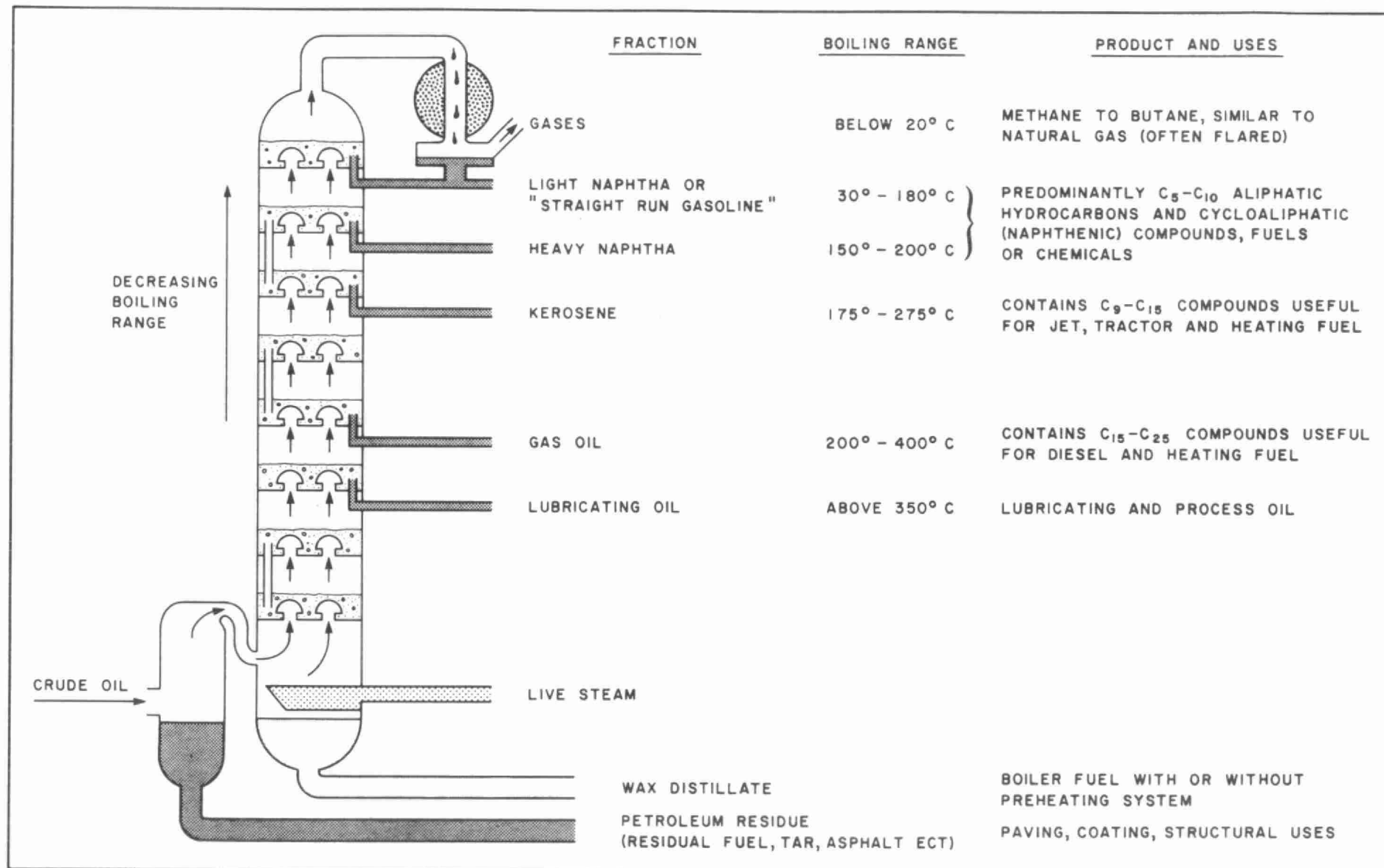


FIGURE 1 — FRACTIONATION COLUMN FOR PETROLEUM DISTILLATION

\* 10% ST 2100 ON 20/100 SUPELCOPORT

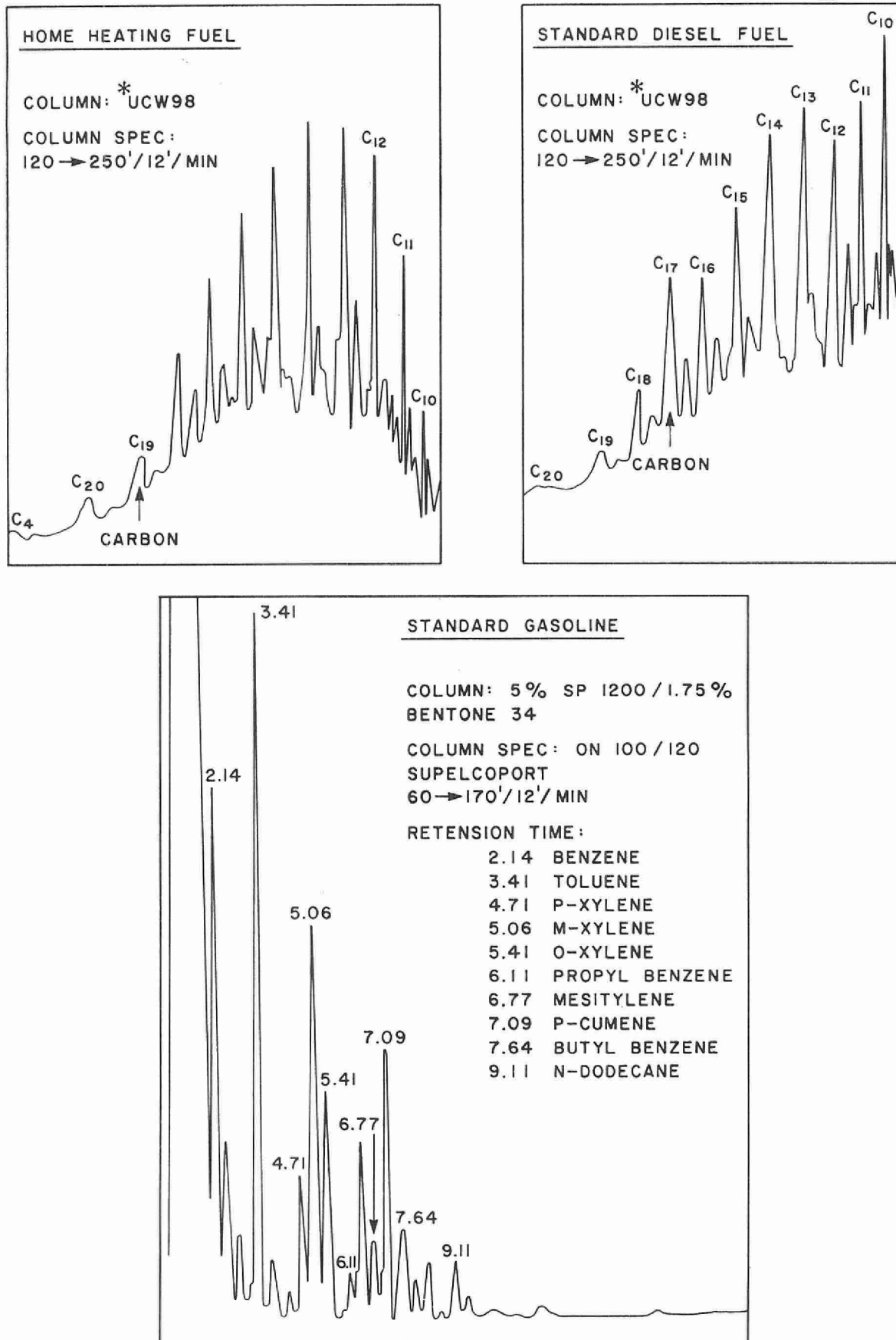


FIGURE 2 — TYPICAL CHROMATOGRAMS OF VARIOUS FUEL OIL AND GASOLINE

## THE DETERMINATION OF pH

The measurement of hydrogen ion activity, commonly referred to as pH, is frequently used in assessing water quality. pH is defined as the negative logarithm of the hydrogen ion activity and is expressed in logarithmic units. Hence it is important to realize that a pH change of one unit (constitutes) a tenfold change in hydrogen ion concentration.

Many chemical and biochemical reactions occur only at a specific pH or within a narrow pH range. Therefore, pH determinations provide a rapid estimate of the activity of these reactions within the water or soil sample. The toxicity of many substances is altered by changes in pH. For example, an ammonia concentration harmless to fish at neutral pH can be lethal at a higher pH. Similarly, iron and aluminum which may be fixed at a higher pH may be readily soluble and toxic to some plants at low pH levels. Most organisms thrive only within a narrow pH range. Consequently, pH may control the type and number of organisms, thereby affecting the rate, character, and occurrence of basic biochemical processes. pH is also of practical importance in acid precipitation studies, flocculation processes, drinking water clarification, the control of anaerobic processes in sewage sludge and the determination of lime requirements for agricultural soils.

Natural waters unpolluted by acid or alkaline wastes generally vary in pH from about 5.0 (acid peat bogs) to 8.5 (chalk streams and marl lakes). Intense photosynthesis results in carbon dioxide removal and can lead to an even higher pH. Most surface waters are protected from strong fluctuations in pH by a natural carbon dioxide-bicarbonate buffering system with the amount of bicarbonate dictating the degree of protection. The pH of soils varies greatly depending on parent material, vegetation and climate.

### Sample Handling and Preservation

#### Water

Glass or plastic bottles are suitable. Bottles should be almost completely filled, tightly closed, refrigerated and delivered promptly to minimize carbon dioxide losses and the effects of biological activity.

Preservatives must not be added, as these will alter the pH of the sample.

#### Soils and Sediments

Plastic containers or glass jars are suitable for sample collection. No preservatives should be added.

### Selection of Method

Measurement of the hydrogen ion activity is performed on waters, soils and sediments by means of a hydrogen ion sensitive glass electrode in conjunction with a suitable reference electrode and millivoltmeter.

The colorimetric determination of pH using indicators is subject to many interferences and shortcomings when used in natural waters, and provides only approximate pH values.

The pH of a soil or sediment can be determined by making a slurry with a portion of sample and either water or 0.01M calcium chloride. Although traditionally, measurements have been made in water, measurement in calcium chloride is preferred for a number of reasons. The value obtained is almost independent of dilution. Soil suspensions are flocculated and therefore errors due to liquid junction potential can be minimized by placing the calomel electrode in the supernatant liquid. The 0.01 M  $\text{CaCl}_2$  solution is more closely equivalent to the total electrolyte concentration of the soil solution of a non saline soil at optimum field conditions. The measurement of pH in 0.01M  $\text{CaCl}_2$  more closely represents a soil solution in equilibrium with atmospheric carbon dioxide and this gives a truer measure of pH in calcareous soils.

## pH

### Electrometric Method

#### SUMMARY

<b>Matrix.</b>	pH is determined on drinking and surface waters, precipitation, sewage, industrial effluent and soil and sediment samples.
<b>Substance determined.</b>	Hydrogen ion activity. At high pH levels (low hydrogen ion concentrations), the activity closely approximates the hydrogen ion concentration.
<b>Interpretation of results.</b>	Results are reported in pH units (the negative logarithm of hydrogen ion activity). A pH of 7 is neutral. The lower the pH, the more acidic a solution becomes, and the higher the pH, the more alkaline it becomes. A change of 1 pH unit represents a tenfold change in the hydrogen ion activity.
<b>Principle of method.</b>	The potential of an electrode chain consisting of a hydrogen ion sensitive glass electrode and a reference electrode, both immersed in the solution under test, is measured on a millivoltmeter calibrated in pH units against buffers of known pH. For soil and sediment samples the preferred test solution is a slurry of soil and 0.01M calcium chloride although soil and distilled water may also be used.
<b>Time required for analysis.</b>	A single water sample measurement requires 1 - 3 minutes. Approximately 150 measurements can be completed in 1 working day. Soil and sediment samples may require a greater length of time to equilibrate.
<b>Range of application.</b>	This method may be applied to samples over the pH range of 0 - 14. The range within which the majority of the samples fall depends to a large extent on the sample matrix. Precipitation samples usually fall within the pH range of 3.5 to 7.0; river and lake samples vary between pH 4 and 9, drinking water usually has a pH between 6.9 and 9.0, sewage and industrial waste samples vary between pH 7.0 and 9.0 and soil and sediment samples usually vary between 4.0 and 9.0.
<b>Standard deviation.</b>	Standard deviations in pH units for the 20 - 50% segments of the ranges defined above are as follows: precipitation - 0.022; rivers and lakes - 0.0315; drinking water - 0.023 and sewage and industrial wastes - 0.0277, soils - .04.
<b>Accuracy.</b>	Recoveries of two buffers (6.86 and 4.008) were 100%.

**Detection  
criteria.**

Not applicable.

**Interferences and  
shortcomings.**

For water samples, sodium ion concentrations in excess of 0.1M will cause a measurable negative error in pH readings greater than 10. A correction may be made using sodium error graphs supplied by the manufacturer for each electrode. "Low sodium ion error" electrodes are available where this problem is frequently encountered. For soil and sediment the salt effect can be overcome, or rather standardized by measurement of pH in 0.01  $\text{CaCl}_2$ . Coating of the glass membrane by colloids, oils, fats, dirt, etc. will result in a sluggish response. Cleaning instructions usually accompany the electrodes or are given in the manufacturer's instruction manual. Free HF in any solution will attack and permanently damage glass components. The loss of  $\text{CO}_2$  during measurement, especially in lightly buffered systems, can result in appreciable pH drift. There may be problems obtaining a steady pH reading when low conductivity samples are analyzed.

**Minimum volume  
of sample.**

50 ml for water samples, 10 g for soil and sediment samples.

**Preservation and  
sample container.**

No preservatives are used. Glass or plastic containers are suitable for water samples. Cap containers tightly, refrigerate and ship immediately. Soil and sediment samples are collected in glass jars.

**Safety  
considerations.**

Normal laboratory precautions for handling samples.

## pH

### Electrometric Method

#### 1. Introduction

pH is defined as the negative logarithm of the hydrogen ion activity:  
 $\text{pH} = -\log(a_{\text{H}^+})$ .

At low hydrogen ion concentrations, hydrogen ion activity closely approximates the hydrogen ion concentration ( $a_{\text{H}^+} \approx (\text{H}^+)$ ).

Therefore  $\text{pH} \approx -\log (\text{H}^+)$

pH is measured directly as the millivolt potential established in an electrode chain consisting of a hydrogen ion sensitive glass electrode and a calomel reference electrode immersed in the solution under test. The millivoltmeter is calibrated with buffers of known pH.

At 25°C, a pH of 7 is defined as neutral. The lower the pH, the more acidic a solution becomes, and the higher the pH, the more alkaline it becomes.

#### 2. Interferences and Shortcomings

At sodium concentrations above 0.1 molar, pH measurements above 10 become subject to sodium ion error. A correction for standard electrodes can be obtained from the manufacturer's literature. Low sodium error electrodes can be obtained for applications where this problem is frequently encountered. For soil and sediment samples errors due to dilution and the liquid junction potential are minimized and the salt effect can be standardized by measurement of pH in 0.01M  $\text{CaCl}_2$ . The use of calcium chloride also provides a truer measure of calcareous soil and sediment pH under normal field conditions since the pH of the calcium chloride ( $\approx 5.7$ ) is similar to the pH of rainwater in equilibrium with atmospheric carbon dioxide at 25°C. In the presence of free carbonates, pH measurements in distilled water and in  $\text{CaCl}_2$  are affected by the carbon dioxide partial pressure.

Loss of  $\text{CO}_2$  from the sample during measurement will cause considerable drift in the readings, especially when low buffer (low ionic content) samples are under test. High quality, properly maintained electrodes and meter will generally respond quickly enough to minimize this problem.

Coating of the glass membrane by organic substances or dirt particles will cause sluggish response of the electrode, and may cause permanent damage. Organic material may be removed with a little acetone, followed by distilled water. Careful blotting with a soft tissue is tolerable. Do not allow the electrode to become thoroughly dried out.

There may be problems obtaining a steady pH reading when low conductivity samples are analyzed.

Free hydrogen fluoride (HF) will attack all glass parts of the electrode system, and will cause rapid and permanent deterioration of the glass membrane surface.



### 3. Apparatus

- 3.1. pH meter, Radiometer or Fisher Accumet type or equivalent.
- 3.2. Glass electrode and calomel reference electrode, or a combination electrode (Ingold electrode number 401-80-LOT recommended for precipitation samples).
- 3.3. Magnetic stirrer and stirring bars.
- 3.4. Beakers, 50 ml or disposable plastic cups (for soil and sediment samples only).
- 3.5. Berzelius beakers, 200 ml, high form (for precipitation samples).

NEW ELECTRODES MUST BE TREATED ACCORDING TO THE MANUFACTURER'S SPECIFICATION BEFORE BEING USED. REFER TO THE LITERATURE OR TO THE pH METER INSTRUCTION MANUAL FOR SPECIAL METHODS OF CLEANING AND RECONDITIONING USED ELECTRODES, AND FOR GENERAL ELECTRODE MAINTENANCE PROCEDURES.

### 4. Reagents

- 4.1. Standard buffer solutions: pH 6.86 or 7.00; pH 4.00 and pH 9.00.
- 4.2. Saturated Potassium Chloride (KCl) solutions.
- 4.3. Acetone.

For Precipitation Samples Only:

- 4.4. Potassium hydrogen phthalate ( $\text{COOH.C}_6\text{H}_4\text{.COOK}$ ), primary pH standard grade powder.
- 4.5. Potassium chloride (KCl) reagent grade crystals.
- 4.6. Potassium phosphate monobasic - disodium phosphate, pH 6.86, dry buffer, Fisher.
- 4.7. **Quality Control Solutions**

#### 4.7.1. Potassium Hydrogen Phthalate Solution (pH 4.008 at 25°C)

Dissolve 20.4230 g potassium hydrogen phthalate (oven dried at 105°C for at least 2 hours) in to 2 liters of distilled, deionized water that has been purged with an inert gas for about 30 minutes.

#### 4.7.2. Phosphate Buffer Solution (pH 6.86)

Prepare according to directions on package. Make up in 2 liter volumes.

For Soils and Sediments Only:

- 4.8. Hydrochloric acid (HCl) concentrated, reagent grade.
- 4.9. Calcium Hydroxide  $\text{Ca(OH)}_2$ , reagent grade powder.

4.10. Calcium chloride ( $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ ), reagent grade powder.

4.11. Calcium Chloride Stock Solution (3.6M)

Dissolve 1059 g calcium chloride in distilled water and dilute to 2 liters.

4.12. Calcium Chloride Working Solution (0.01M)

Dilute 50 ml stock solution to 18 liters with distilled water. The pH should be between 5.0 and 6.5; if it is not, adjust with either calcium hydroxide or hydrochloric acid as required.

5. Procedure

REFER TO MANUFACTURERS' MANUALS FOR OPERATION AND MAINTENANCE OF THE pH METER, AND CARE OF THE ELECTRODES.

5.1. pH Determination on Water, Sewage and Industrial Waste Samples.

5.1.1. Turn the pH meter on with the function switch in the standby position. Allow a few minutes to warm-up.

5.1.2. Check that the calomel reference electrode potassium chloride filler hole is clear, and that the saturated potassium chloride level in the electrode is maintained above the level of the samples during measurement. Potassium chloride crystals must be present at all times, however only a few crystals are required, as overloading will cause crystals to clump together resulting in slow electrode response.

5.1.3. Set the temperature compensation control to the temperature of the buffers and samples to be analyzed. All samples and buffers must be allowed to equilibrate at the same temperature before analysis.

5.1.4. Rinse electrodes with distilled water. Rinse sample cup or beaker, stirrer bar and electrodes with sample portion of buffer used for setting meter. Discard buffer and refill cup with a fresh quantity. Lower electrode(s) into sample, start stirrer and allow meter needle or digital display to stabilize before proceeding.

5.1.5. Adjust meter to read correct pH using the appropriate control. Based on the routine working ranges for the different sample matrices, the following buffers are used:

Drinking water,  
sewage and  
industrial waste:

Set pH meter on 6.86 buffer solution and read 9.00 and 4.00 buffers as QC-A and QC-B respectively.

River and lakes:

Set pH meter on 4.00 and 9.00 buffer solutions and read 8.00 and 6.86 buffer solutions as QC-A and QC-B respectively.

Precipitation:

Set pH meter on 7.00 and 4.01 buffer solutions and read 6.86 buffer and 4.008 (potassium hydrogen phthalate solution) as QC-A and QC-B respectively.

- 5.1.6. Repeat the procedure as outlined in step 5.1.4., using the sample under test instead of the buffer solution. Samples should be at the same temperature as the buffer solutions. Samples are read to the nearest 0.01 pH unit. Duplicates should be run at the beginning and end of the run.

**NOTE:** A slow response time (greater than one minute) implies that the electrode is deteriorating. Clean the electrode. If this does not improve the response time, replace the electrode.

**NOTE:** For precipitation samples of small volume (less than 100 ml), the degree of mixing cannot be adequately controlled when samples are stirred. In this case the pH system must be calibrated and the samples measured without stirring. pH responses must be recorded after 60 seconds but before 120 seconds to prevent polarization which becomes pronounced after 2 minutes.

- 5.1.7. When all measurements have been completed, rinse the electrodes thoroughly with distilled water. Inspect the electrodes for dirt or grease contamination and clean thoroughly if necessary. Place the electrodes in a beaker of pH 6.86 or pH 7.00 buffer solution (or other solution as recommended by the manufacturer). Do not store combination electrode used for precipitation samples in buffer solutions. Store in distilled water or in dilute hydrochloric acid ( $\approx$ pH 2)
- 5.1.8. If electrode has been stored in distilled water or used constantly for low conductivity samples (e.g. precipitation samples), the glass membrane may become dehydrated resulting in a sluggish unstable response. Soaking in dilute hydrochloric acid ( $\approx$ pH 2) for 2 hours, followed by soaking in 6.86 or 7.00 buffer for 1 hour will usually remedy this problem.
- 5.1.9. When using the Radiometer PHM64 meter the sensitivity control setting should always be in the range of 98 - 100%.

## 5.2. Determination of pH in Soils and Sediments ( $\text{CaCl}_2$ ).

- 5.2.1. Place about 10 g of soil into a 50 ml beaker or a disposable paper or plastic cup. Add about 20 ml 0.01M calcium chloride solution and stir suspension several times during the next 30 minutes (distilled water is used instead of  $\text{CaCl}_2$  if comparisons are to be made with historical data). For organic soils which absorb all the solution use a 4:1 solution:soil ratio.
- 5.2.2. Let suspension stand for 30 minutes and allow most of the sediment to settle. During this time calibrate the pH meter as described in 5.1.1 - 5.1.5. Ensure that the buffers used for calibration have a pH at the lower and upper end of the pH range of the soils being measured.

**NOTE:** It may be advantageous to test the pH of the soil or sediment with multi-range pH paper prior to pH determination on the pH meter. This will give an idea of the pH range of the samples under investigation and allow the rapid determination of the buffers most suitable for calibration of the meter.

- 5.2.3. Measure pH of sample by immersing the glass electrode into the partially settled suspension (do not allow electrode to touch the bottom of container) and placing the calomel electrode in the clear supernatant solution. If a combination electrode is used, immerse in supernatant solution. Record pH.

5.3. Determination of pH in Soil and Sediment (Water)

- 5.3.1. Place 20 g soil or sediment in a 50 ml beaker or plastic cup. Add 20 ml distilled water and stir suspension during the next 30 minutes. For organic samples used a 1:2 or a 1:4 soil:water ratio.
- 5.3.2. Allow suspension to settle for 30 minutes.
- 5.3.3. Measure pH as outlined in 5.2.3.

6. Calculation and Reporting

River, domestic water, sewage and precipitation samples are read to the nearest 0.01 pH unit while soil samples are read to the nearest 0.1 pH unit.

7. Precision and Accuracy

Recoveries of two buffers (6.86 and 4.008) are 100%. Accuracy measurements on soil and sediment samples have not been made.

Standard deviations of water, sewage and industrial waste samples are as follows:

Sample	Routine operating range	$S_{md}$	$S_{ld}$	$S_{hd}$
Precipitation	3.5 - 7.0	0.022	-	-
River and lakes	4 - 14	0.0315	0.026	-
Drinking water	6.9 - 9.0	0.023	-	-
Sewage, industrial waste	7.0 - 9.0	0.0277	-	-
Soils	3.5 - 8.0	0.04	-	0.1

Where:

$S_{md}$  = standard deviation of mid-level within run duplicates (20 - 50% of routine operating range).

$S_{ld}$  = standard deviation of low-level within run duplicates (0 - 20% of routine operating range).

$S_{hd}$  = standard deviation of high-level within run duplicates (50 - 100% of routine operating range).

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## THE DETERMINATION OF PHENOLIC COMPOUNDS

Phenolic compounds are present in the aquatic environment as a result of natural and industrial processes. Decomposition of lignin and degradation of biocides and pesticides produce a variety of phenolics. Significant quantities of phenolic type compounds are by-products of the steel industry, petrochemical and petroleum industries, pulp and paper processors, plastic manufacturers and associated chemical industries.

Phenolic compounds are potentially toxic to humans and are readily absorbed directly through the skin, rapidly concentrating in the kidneys, liver, and intestines. Chlorination of drinking water supplies and treatment of wastewaters containing phenolics, produces chlorinated phenols which are often responsible for taste and odor problems. Taste and odor thresholds at phenol concentrations of 6 - 8  $\mu\text{g/l}$  have been reported. Phenolic compounds can also affect aquatic biota by direct toxicity.

Although phenolics are bactericides at moderate or stronger concentrations, some weak phenolic solutions are readily decomposed by bacterial or biological activity. *Pseudomonas* are known to degrade many phenolic compounds (8.1), however, polyhalogenated derivatives and nitrosubstituted phenols are much more resistant to bacteriological attack and may persist for months (8.4) in receiving water. Biological oxidation in activated sludge tanks is generally effective for removing phenolics at sewage treatment plants. Phenolic removal processes at water treatment plants include super-chlorination, chlorine dioxide, ozonation, and activated carbon absorption (8.2).

### Sample Handling and Preservation

Samples for phenolics analysis are usually collected in glass bottles and preserved with a mixture of copper sulphate acidified with phosphoric acid. This inhibits biodegradation or chemical oxidation of the sample during shipment or storage at the laboratory, thereby preventing significant losses of phenolics. Plastic containers and bakelite screw caps should be avoided as there is a strong tendency for phenolic confirmation from the walls of the container and screw caps.

### Selection of Method

Absorptiometric, fluorescence and gas chromatographic methods have been used for the determination of phenol and various substituted phenolics. Although gas chromatographic methods are capable of determining specific phenolic compounds, the sample preparation procedure and the interpretation of individual chromatograms does not lend itself to routine analysis. Furthermore the sensitivity is not adequate for most routine samples. Ultraviolet and infrared methods also recover all types of phenols; however, these methods may not be sensitive enough. Fluorescence methods are very sensitive to most substituted phenols except chlorophenols (8.3) but they require lengthy clean-up

procedures. Colorimetric methods do not permit a reaction with all derivatives of phenol and only partially recover others. However, colorimetric methods are extremely sensitive and capable of measuring phenolic compounds at the 1 µg/l phenol level.

The 4-aminoantipyrine (4-AAP) automated colorimetric method (Method A) with an automated distillation step is used for routine river, lake, drinking water and sewage samples. Various manual pretreatment techniques are used for industrial type samples when large amounts of organics and/or petroleum hydrocarbons are present. Appendix A details the distillation procedure which is applied to samples that may contain interferences other than petroleum products. Appendix B describes the extraction and distillation procedure for samples containing petroleum products. Appendix C pertains to samples for which interferences are detected by the method in Appendix A. The Gibb's method is often used to obtain the concentration range prior to analysis by the 4-AAP Method.

## PHENOLIC COMPOUNDS

### Automated 4-Aminoantipyrine Method A

#### SUMMARY

<b>Matrix.</b>	This method is used for routine river, lake, drinking water and sewage samples and for some industrial waste samples.
<b>Substance determined.</b>	Phenol and substituted phenolics other than those para substituted phenols in which the substitution is alkyl, aryl, nitro, benzoyl, nitroso, or aldehyde group.
<b>Interpretation of results.</b>	Phenol is used as a standard for the colorimetric procedure. Any color produced by the reaction of other phenolics is reported as phenol. Since the recovery of phenolics is non-uniform, substitution usually reduces recovery and the reported value represents the minimum concentration of phenolic compounds present in the sample.
<b>Principle of method.</b>	After distillation of the sample from an acidic medium to remove interferences, the distillate is buffered to pH 9.4 and reacted with 4-aminoantipyrine. In the presence of an alkaline oxidizing agent, potassium ferricyanide, a red antipyrine dye is formed and measured colorimetrically at 505 nm.
<b>Time required for analysis.</b>	With a sampler rate of 20 analyses per hour about 150 tests per day may be processed.
<b>Range of application.</b>	0.3 - 50 $\mu\text{g/l}$ phenol. Higher levels are determined by dilution.
<b>Standard deviation.</b>	Based on within run duplicates, standard deviations are: 0.175 $\mu\text{g/l}$ for 0 - 20% of the range and 0.744 $\mu\text{g/l}$ for 20 - 50% of the range.
<b>Accuracy.</b>	Recoveries for two Quality Control standards were 99.6% and 99.3%.
<b>Detection criterion.</b>	0.29 $\mu\text{g/l}$ phenol.



**Interferences  
and shortcomings.**

Potential colorimetric interferences are removed during automated distillation. Ferrous sulphate should be added to samples known to contain oxidizing agents. Manual solvent extraction and distillation techniques may be required for oil and tar laden samples. Certain para substituted phenolics do not react with 4-AAP, therefore analysis represents a minimum phenolic concentration.

**Minimum volume  
of sample.**

150 ml of preserved sample.

**Preservation and  
sample containers.**

Special glass sample bottles containing copper sulphate and phosphoric acid are provided by the laboratory. Unpreserved samples lose phenolics in storage. Due to the corrosive nature of the preservatives, sample bottles should be stored in an upright position to prevent acidic corrosion of foil liners in bottle caps.

**Safety  
considerations.**

The preservative solution is strongly acidic. Flush any spilled preservative with copious amounts of water. Phenolic standards are prepared from pure phenol which is extremely hygroscopic and can be absorbed through the skin. Phenol crystals and burns should be flushed with methanol (keep away from eyes) followed by large amounts of cold water.

## PHENOLIC COMPOUNDS

### Automated 4-Aminoantipyrine Method A

#### 1. Introduction

An unfiltered sample aliquot enters the AutoAnalyzer system where it is mixed with phosphoric acid prior to entering the automated distillation system. The distillate is mixed with a tartrate-borax buffer, pH 9.4, and 4-AAP to produce an antipyrine dye which is oxidized by alkaline ferricyanide. The absorbance of the red antipyrine dye is measured colorimetrically in a 5 cm flow cell at 505 nm. The result in  $\mu\text{g}$  phenol/l is read from the chart recorder trace by comparison with peaks produced by a similarly treated series of standards.

The term "phenolic compounds" is applied here to those hydroxy derivatives of benzene which react under the conditions of the tests, with the reagents used. The percentage composition of the phenolic compounds present in a given sample is unpredictable. A standard mixture could not, therefore, be applicable to all samples. For this reason phenol is used as standard and any color produced by reaction with the reagent is reported as phenol.

#### 2. Interferences and Shortcomings

Most colorimetric interferences are removed by distillation of samples. Oils, greases, and tars, however, may also distil and therefore a manual extraction procedure is available to obtain a homogeneous clear sample for analysis. Oxidizing agents present in the original sample should be removed by adding ferrous sulphate or sodium arsenite at the time of sampling.

Phenol-free distilled water must be used to detect minimum levels of phenolics. Many para substituted phenolics cannot react with 4-AAP since the reaction product cannot be formed unless the para substituted group is eliminated. Results obtained by this procedure represent the minimum concentration of phenolic compounds present in the sample.

#### 3. Apparatus

3.1. AutoAnalyzer AAI System consisting of the following modules:

- 3.1.1. sampler
- 3.1.2. proportioning pump
- 3.1.3. heating bath with distillation head and modified condensing system.
- 3.1.4. colorimeter equipped with 505 nm filters and a 5 cm flow cell

- 3.1.5. voltage regulator
- 3.1.6. range expander
- 3.1.7. chart recorder
- 3.2. Pump tubing manifold and associated manifold glassware as shown in Figure 1.
- 3.3. Culture tubes, 25 x 100 mm.
- 3.4. Culture tube racks, 40 tube capacity.
- 3.5. Dilution tubes, 50 ml capacity.
- 3.6. Reagent bottles, light restricting.
- 3.7. Reagent bottles, 1 liter.
- 3.8. Reagent reservoir bottles, 9 liter.

#### 4. Reagents

- 4.1. Sodium hydroxide (NaOH), reagent grade pellets.
- 4.2. Ethylenediaminetetra-acetic acid, disodium salt (EDTA)  
( $\text{CH}_2\text{N}(\text{CH}_2\text{COOH})_2\text{CH}_2\text{COONa})_2 \cdot 2\text{H}_2\text{O}$ ), reagent grade powder.
- 4.3. Phenol ( $\text{C}_6\text{H}_5\text{OH}$ ), reagent grade crystals.
- 4.4. Acetone ( $\text{C}_3\text{H}_8\text{O}$ ), reagent grade.
- 4.5. Phosphoric acid ( $\text{H}_3\text{PO}_4$ ), reagent grade, concentrated (85%).
- 4.6. Cupric sulphate, 5 hydrate ( $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ ), reagent grade crystals.
- 4.7. di-Sodium tetraborate ( $\text{Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O}$ ), reagent grade crystals.
- 4.8. Sodium potassium tartrate ( $\text{NaKC}_4\text{H}_4\text{O}_6 \cdot 4\text{H}_2\text{O}$ ), reagent grade crystals.
- 4.9. 4-Aminoantipyrine, ( $\text{CH}_3\text{CC}(\text{NH}_2)\text{CON}(\text{C}_6\text{H}_5)\text{NCH}_3$ ), reagent grade powder.
- 4.10. Potassium ferricyanide ( $\text{K}_3\text{Fe}(\text{CN})_6$ ), reagent grade powder.

#### 4.11. Phosphoric Acid Solution

In a volumetric flask, add 100 ml of 85% phosphoric acid to approximately 800 ml of distilled, deionized water. Dilute to 1 liter when cool.

#### 4.12. Buffer Solution

In a volumetric flask, add 50 g di-sodium tetraborate and 25 g sodium potassium tartrate to approximately 900 ml of distilled, deionized water. Add 10 ml acetone and stir until dissolved. Dilute to 1 liter and filter if necessary.

#### 4.13. 4-AAP Solution

In a volumetric flask, dissolve 0.20 g of 4-aminoantipyrine in 200 ml of deionized, distilled water and transfer to a darkened storage bottle before use. Prepare a fresh solution daily.

#### 4.14. Potassium Ferricyanide Solution

In a volumetric flask, dissolve 1.0 g of potassium ferricyanide in deionized, distilled water and dilute to 200 ml. Transfer to a darkened storage bottle before use. Prepare a fresh solution daily.

#### 4.15. Copper Sulphate-Phosphoric Acid Preservative

Carefully and with continuous stirring add 50 ml of concentrated phosphoric acid to approximately 900 ml of phenol-free distilled water. Dissolve 96 gm of copper sulphate in deionized, distilled water and after dissolution is complete, dilute to a final volume of 1 liter.

#### 4.16. Phenol Stock Solution (1000 mg/l)

Dissolve 1.00 g reagent grade phenol crystals in phenol-free distilled water and dilute to 1 liter in a volumetric flask. Mix well and store in a tightly stoppered container.

**NOTE:** Phenol is an acid, highly toxic, and readily absorbed through the skin. In case of contact, wash the affected area continuously with water and soap (or mild detergent) for at least five minutes.

#### 4.17. Phenol Intermediate Solution (1000 µg/l)

Transfer a 1.0 ml aliquot of the concentrated stock standard phenol solution to a 1 liter volumetric flask containing approximately 800 ml of distilled water. Dilute to 1 liter with phenol-free distilled water. Mix well before using. Prepare a fresh solution daily.

#### 4.18. Phenol Working Solution (25 µg/l) (Calibration)

Transfer a 25 ml aliquot of the phenol intermediate solution to a 1 liter volumetric flask containing approximately 800 ml of phenol free distilled, deionized water. Add 5 ml of the copper sulphate solution and dilute to 1 liter. Mix well before using. Prepare fresh solution daily.

#### 4.19. Quality Control Solutions

A quality control stock solution is prepared as in 4.16 using a different batch of phenol. A quality control intermediate solution is then prepared from this stock solution as described in 4.17. Quality control A and B solutions (QC-A and QC-B) are prepared to cover the calibration range of the system. To each

liter of solution, 5 ml of the preservative is added. A long term blank (LTBI) is also prepared by diluting 5 ml of the preservative to 1 liter with phenol free distilled, deionized water. Store at 5°C to minimize biological activity.

Prepare these solutions to provide for, at least, 20 days of analysis. Introduce new solutions and monitor their concentration at least 3 days prior to their adoption.

#### 4.20. Basic Cleaning Solution (NaOH-EDTA)

Cautiously and with continuous stirring, dissolve 250 g of reagent grade sodium hydroxide pellets in approximately 1000 ml of distilled water. Add approximately 60 g of ethylenediaminetetra-acetic acid, disodium salt.

**NOTE:** EYE PROTECTION MUST BE WORN WHEN HANDLING SODIUM HYDROXIDE AS IT IS EXTREMELY CAUSTIC AND MAY CAUSE SEVERE BURNS TO UNPROTECTED AREAS.

#### 4.21. Sample Containers for Phenols Analysis

Precharged sample containers (170 ml Prince of Wales bottles with foil lined caps ) are provided by the laboratory for phenol samples. Add 1.0 ml of the copper sulphate-phosphoric acid solution to each sample bottle and store containers in an upright position.

### 5. Procedure

REFER TO MANUFACTURER'S MANUAL FOR CLEANING, SET-UP AND CHECKING PROCEDURES FOR AUTOANALYZER SYSTEM.

- 5.1. After collecting the samples, group samples by classification.
- 5.2. Rinse each sample tube at least twice with 15 ml portions of sample prior to accepting a third portion for analysis.
- 5.3. Place the culture tube containing the sample into a test tube rack.
- 5.4. Set up AutoAnalyzer as in Figure 1.
- 5.5. Load samples into sampler. Each run of samples will include all of the following units: distilled water blank (BI); quality control solutions (LTBI, A and B); high sensitivity check (H); samples in groups of 10 or less.  
Load samples in the following sequence:  
H; BI; H H; BI; LTBI; A; B; n(10 samples; BI; 10 samples; H; BI)  
Where n is the number of repetitions of the bracketed sequence required to accommodate the workload.
- 5.6. Due to the perishable nature of this parameter, samples should not be accumulated before running, but should be analyzed as they are received.
- 5.7. If the blanks and quality control checks agree with previous results, record the measured values of the LTBI, QC-A, QC-B, and the STD. CAL. setting during

the run. Also, record the values of the Highs and determine if a within run sensitivity correction should be applied.

5.8. Read and record each sample peak using the calibration chart.

## 6. Calculation and Reporting

Multiply the reading by the dilution factor

$$DF = \frac{\text{diluted volume}}{\text{aliquot volume}}$$

and record the result. Report the results according to the following schedule:

High and Low range:

A maximum of 2 significant figures are shown when reporting to 1 decimal place.

## 7. Precision and Accuracy

Precision - duplicate analyses of routine samples

Sample Concentrations ( µg/l as Phenol)	Standard Deviation ( µg/l as Phenol)
40	0.175
10 - 25	0.744
25 - 50	-

Accuracy - recovery of quality control (QC) solutions

QC Concentration ( µg/l as Phenol)	Average Recovery ( µg/l as Phenol)	Standard Deviation ( µg/l as Phenol)
25.00	24.89	0.539
10.00	9.93	0.342

## 8. Bibliography

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- 8.4. Wiebe, J.D. Jr. (1975). Phenols in Water, Reply to OECD Questionnaire. (monograph). Canada Centre of Inland Waters, Department of the Environment, Burlington, Ontario.

## PHENOLIC COMPOUNDS

### APPENDIX A

#### Distillation Procedure

##### 1. Introduction

Phenols are separated from non volatile materials by distillation at a more or less constant rate. The rate of volatilization is gradual and the volume of the distillate must equal the volume of sample being distilled. Acidity, during distillation, must be maintained in order to prevent precipitation of cupric hydroxide which could oxidize the phenols present.

##### 2. Interferences and Shortcomings

If the distillate is turbid after distillation or if a sample is suspected of containing petroleum products then an second aliquot of sample is extracted (see Appendix B and C) before distillation.

##### 3. Apparatus

3.1. All pyrex distillation apparatus, consisting of:

3.1.1. distillation flask, 1000 or 500 ml.

3.1.2. separatory funnel, 250 ml.

3.1.3. T joint side arm

3.1.4. Graham condenser.

3.1.5. gas burner.

3.2. graduated cylinders, 100 and 500 ml.

##### 4. Reagents

Prepare all reagents with distilled, deionized water free of phenols.

4.1. Lead carbonate,  $\text{PbCO}_3$  reagent grade.

4.2. Sodium arsenite,  $\text{NaAsO}_2$  reagent grade.

4.3. Methyl Orange indicator.



#### 4.4. Methyl Orange Indicator Solution

Dissolve 0.1 g methyl orange in 100 ml distilled water.

#### 4.5 Phosphoric acid ( $\text{H}_3\text{PO}_4$ ), reagent grade, concentrated (85%).

### 5. Procedure

- 5.1. Take a 100 ml aliquot of sample in a graduated cylinder and acidify with concentrated phosphoric acid, using methyl orange indicator, to around pH 4.0 (If a smaller aliquot is needed dilute the aliquot to 100 ml with distilled water.)
- 5.2. Sodium arsenite (0.1 g) is added if the presence of oxidizing agents, such as chlorine, is suspected. Sulphides may be precipitated by the addition of lead carbonate (0.5 g).
- 5.3. Transfer the sample aliquot to the distillation flask and attach to the Graham condenser. Boiling chips or glass beads are used to minimize bumping. A few drops of an antifoam agent may be added if it is suspected that the sample may foam upon heating. Distilled water is added to the separatory funnel, 10 ml for 100 ml sample size.
- 5.4. The cylinder used to measure the sample aliquot is rinsed with distilled water and used as a receptacle for the distillate.
- 5.5. Distill 90% of the volume of the sample aliquot and stop the distillation. Allow to cool a few minutes then add the distilled water quickly from the separatory funnel. Continue the distillation until a volume equal to the aliquot (100 ml) has been distilled.
- 5.6. Remove the distillate receiver, mix well and reserve for analysis by Method A
- 5.7. Use 25 ml of the distillate to estimate the phenolic concentration by the Gibbs method if desired.

#### **NOTE:** PROCEDURE FOR CLEANING THE DISTILLATION FLASK.

Wash the flask with hot water. If a grease deposit is observed, add 10% potassium hydroxide to the flask and heat gently with swirling.

**SAFETY:** SAFETY GLASSES SHOULD BE WORN DURING THIS OPERATION. CARE SHOULD BE TAKEN TO AVOID BUMPING.

Wash out the flask and half fill with distilled water. Attach to the Graham condenser and steam out, by heating the flask with no water running through the condenser, for 10 - 15 minutes. Cool and discard the water from the distillation flask.

## PHENOLIC COMPOUNDS

### APPENDIX B

#### Pre-screening procedure for samples contaminated with petroleum products.

##### 1. Introduction

The sample is made alkaline (pH >12) with potassium hydroxide. The sample is then extracted with chloroform to isolate the phenolates from other organics. After driving off any chloroform which remains, the sample is acidified with phosphoric acid to the methyl orange end point. The sample is now ready for distillation.

##### 2. Interferences and Shortcomings

This technique is used on a multiphase sample containing petroleum products. Any treatment procedure may result in the unavoidable loss of certain types of phenols due to emulsification or incomplete extraction of phenol.

##### 3. Apparatus

- 3.1. beakers
- 3.2. separatory funnels, 250 ml
- 3.3. water bath
- 3.4. graduated cylinders

##### 4. Reagents

Prepare all reagents with distilled water free of phenols.

- 4.1. Chloroform,  $\text{CHCl}_3$ , reagent grade.
- 4.2. Potassium hydroxide, KOH, reagent grade.
- 4.3. **Potassium Hydroxide Solution (10%)**

Dissolve 100 g of potassium hydroxide in 1 liter of distilled, deionized water.

**NOTE:** Safety equipment including eye protection must be worn when handling potassium hydroxide as it is extremely caustic and may cause severe burns to unprotected areas.

## 5. Procedure

- 5.1. Adjust an aliquot (usually 100 ml) of the sample to  $\text{pH} > 12$  with 10% potassium hydroxide.
- 5.2. Transfer the aliquot to a separatory funnel and extract with 25 ml of chloroform.

**NOTE:** RELEASE THE PRESSURE AFTER THE INITIAL SHAKE.

Discard the chloroform phase and repeat the extraction twice more with 25 ml portions of chloroform, discarding the solvent phase.

- 5.3. Transfer the aqueous extract to a 250 ml beaker and heat on the water bath until no chloroform remains (approximately 1 hour). Allow to cool.
- 5.4. Continue with the procedure as described in Appendix A, beginning with step 5.1.

## PHENOLIC COMPOUNDS

### APPENDIX C

#### Pre-screening procedure for samples that have turbid distillates.

##### 1. Introduction

The sample is acidified and extracted with chloroform to collect organic material. The chloroform is then back extracted with a potassium hydroxide solution to isolate the phenolates. After driving off any chloroform which may have remained with the aqueous phase, the aqueous phase is acidified with phosphoric acid to the methyl orange end point. This converts the phenolates back to phenol. The sample is now ready for distillation.

##### 2. Interferences and Shortcomings

This technique is used on single phase samples containing petroleum products or other interferents indicated by Appendix A. Any treatment procedure may result in the unavoidable loss of certain types of phenols due to emulsification or incomplete extraction of phenol.

##### 3. Apparatus

- 3.1. beakers
- 3.2. separatory funnels
- 3.3. water bath
- 3.4. graduated cylinders

##### 4. Reagents

Prepare all reagents with distilled water free of phenols.

- 4.1. Potassium hydroxide, KOH, reagent grade.

##### 4.2. Potassium Hydroxide Solution (1%)

Dissolve 10 g of potassium hydroxide in 1 liter of distilled, deionized water.

**NOTE:** Safety equipment including eye protection must be worn when handling potassium hydroxide as it is extremely caustic and may cause severe burns to unprotected areas.

- 4.3 Phosphoric acid ( $H_3PO_4$ ), reagent grade, concentrated (85%).
- 4.4 Chloroform ( $CHCl_3$ ), reagent grade.

- 4.5 Methyl Orange indicator.
- 4.6 Methyl Orange indicator solution.  
Dissolve 0.1 g methyl orange in 100 ml distilled water.

## 5. Procedure

- 5.1. Acidify an aliquot (usually 100 ml) of the sample with phosphoric acid to about pH 4.0, using methyl orange indicator.
- 5.2. Transfer the aliquot to a separatory funnel and extract with 50 ml of chloroform.

**NOTE:** RELEASE THE PRESSURE AFTER THE INITIAL SHAKE.

Transfer the chloroform phase to a second separatory funnel. Repeat the extraction with 50 ml of chloroform, discarding the aqueous phase after the final extraction and combining the two solvent portions.

- 5.3. Extract the chloroform layer with three portions (50, 25 and 25 ml) of 1% potassium hydroxide, discarding the chloroform into a proper waste container.
- 5.4. Transfer the potassium hydroxide extract to a 250 ml beaker and heat on the water bath until no chloroform remains (approximately 1 hour). Allow to cool.
- 5.5. Adjust the pH of the sample with phosphoric acid using methyl orange indicator. Bring the volume up to 100 ml with phenol free distilled water.
- 5.6. Continue with the procedure described in Appendix A, beginning with step 5.2.



## THE DETERMINATION OF PHENOXYACID TYPE HERBICIDES

Phenoxyacid herbicides (2,4-D type) are widely used for selective weed control in crops that are comparatively resistant to these chemicals and for the control of roadside vegetation. These herbicides may also be used for the control of some aquatic plants. Potential problems occur when agricultural run-off waters containing 2,4-D type residues are used to irrigate a crop which is more susceptible to these residues, thus causing serious damage. These materials can also impart severe taste and odour problems to water supplies at low milligrams per liter levels. Taste problems arise from the ability of the herbicides to decompose to chlorophenols which may be present as impurities in the herbicide formulations.

Although the toxicity of 2,4-D type herbicides to mammals is relatively low, impurities (such as dioxins) which are produced as by-products in the manufacture of these herbicides are extremely toxic.

### Sample Handling and Preservation

#### Water Samples

Samples must be collected in glass bottles due to the possibility of contamination and the low levels of herbicides being investigated. Use 1 liter amber, glass bottles with aluminum foil or Teflon lined screw caps. Bottles are labelled "For Pesticide and PCB Analysis Only". A minimum sample size of 500 ml is required and 800 ml is preferred (level marked on bottle).

### Selection of Method

A routine method for the extraction, methylation, cleanup and determination of 2,4-D type herbicides has been adapted from published methods for use in this laboratory. The identification of herbicides involves gas chromatographic analysis using electron capture detector systems.

## PHENOXYACID TYPE HERBICIDES

### Gas Chromatographic Method

#### SUMMARY

<b>Matrix.</b>	This method is used on water samples.										
<b>Substances determined.</b>	The common 2,4-D type herbicides: <table><tr><td>2,4-D</td><td>Dicamba</td></tr><tr><td>Silvex</td><td>MCPA</td></tr><tr><td>2,4,5-T</td><td>MCP</td></tr><tr><td>2,4-DB</td><td></td></tr><tr><td>2,4-DP</td><td></td></tr></table>	2,4-D	Dicamba	Silvex	MCPA	2,4,5-T	MCP	2,4-DB		2,4-DP	
2,4-D	Dicamba										
Silvex	MCPA										
2,4,5-T	MCP										
2,4-DB											
2,4-DP											
<b>Interpretation of results.</b>	Results are reported in $\mu\text{g/l}$ or other units depending on concentrations. High levels may occur in rural areas during periods of considerable run-off.										
<b>Principle of method.</b>	A sample aliquot is solvent extracted, then dried, concentrated, methylated and cleaned up by Florisil column chromatography to remove interferences. The cleaned up extract is examined by Electron Capture Gas Chromatography. The 2,4-D herbicides are determined as their methyl esters from a standard calibration and peak height quantitation.										
<b>Time required for analysis.</b>	Under optimum conditions, 8-10 water samples may be analyzed for 2,4-D type herbicides in two days.										
<b>Range of application.</b>	From 0.02 $\mu\text{g/l}$ .										
<b>Standard deviation.</b>	Not available.										
<b>Accuracy.</b>	Not available.										
<b>Detection limits.</b>	Detection limits ( $\mu\text{g/l}$ ) are as follows: 2,4-D: 0.05; 2,4-DB: 0.20; 2,4,5-T: 0.025; 2,4-DP: 0.05; Silvex: 0.025; Picloram: 0.05; Dicamba: 0.05.										
<b>Interferences and shortcomings.</b>	Naturally occurring materials may be methylated causing interferences. The EC detector is not specific and can give erroneous results. An Electrolytic Conductivity Detector (Hall) may be used, if available.										



<b>Minimum volume of sample.</b>	800 ml, obtained in specially prepared glass bottles.
<b>Preservation and sample container.</b>	Only 1 liter solvent-rinsed, amber glass bottles are acceptable. Caps should be foil or Teflon-lined to prevent contamination. No preservatives should be used as these will be added on arrival at the laboratory, depending on parameters requested. Samples should be refrigerated at 4°C to avoid bacterial degradation.
<b>Safety considerations.</b>	<p>Solvents pose fire and exposure hazards; extreme care must be taken during their transport, storage and use.</p> <p>In addition to normal solvent precautions, extreme care must be taken in the preparation and storage of diazomethane, which is very toxic. Its preparation should be carried out only in a fume cupboard provided with a powerful exhaust system. The use of a safety glass shield is recommended.</p> <p>Diazomethane is decomposed by rough surfaces; consequently, ground glass joints must be protected by Teflon sleeves. Diazomethane should be stored in a refrigerator in small quantities and should not be kept at room temperature for more than a few hours, as it may decompose suddenly, resulting in an explosion.</p>

## PHENOXYACID TYPE HERBICIDES

### Gas Chromatographic Method

#### 1. Introduction

2,4-D type herbicide residues are extracted from water with an organic solvent. The extracts are dried, evaporated, methylated using diazomethane and cleaned up by adsorption chromatography. Gas chromatographic analysis is performed using an electron capture detector system.

#### 2. Interferences and Shortcomings

Due to the low levels being investigated and the non-specific nature of the EC detector, the presence of small amounts of contaminants may create serious problems. The EC detector, being non-specific, will respond to any material containing any halogenated compounds, or other electron-capturing groups.

#### 3. Apparatus

ALL GLASSWARE MUST BE RINSED THOROUGHLY WITH SOLVENT PRIOR TO USE.

##### 3.1. Extraction Procedure

- 3.1.1. Graduated cylinders, Pyrex, 100 ml, 1 liter.
- 3.1.2. Centrifuge tubes, Pyrex, 15 and 50 ml, graduated, with glass stoppers.
- 3.1.3. Rotary extractor, Buchler.
- 3.1.4. Vortex evaporator, Buchler.
- 3.1.5. Erlenmeyer flask, Pyrex, 500 ml.
- 3.1.6. Filter funnel, Pyrex.
- 3.1.7. Round-bottom flask, Pyrex, 300 ml, with 24/40 glass joint.
- 3.1.8. Rotary evaporator.

##### 3.2. Clean-up Procedure

- 3.2.1. Chromatographic column: Pyrex glass tubing, 28 cm x 6 mm I.D., with Teflon stopcock, 12/30 glass joint at top in which fits a 100 ml Pyrex reservoir.
- 3.2.2. Centrifuge tubes, Pyrex, 50 ml, graduated, with glass stopper.
- 3.2.3. Pasteur pipettes, glass, disposable.

3.2.4. Graduated cylinders, Pyrex, 25, 50 ml.

3.2.5. Kontes blow-down apparatus.

### 3.3. Gas Chromatographic Analysis

3.3.1. Gas chromatograph, Hewlett Packard 5710A, or equivalent, with automated sample injection system.

3.3.2. Column, 50 m, fused silica capillary column with OV101.

3.3.3. Electron capture detector,  $\text{Ni}^{63}$ .

3.3.4. Syringe, Hamilton, 10  $\mu\text{l}$ .

## 4. Reagents

### 4.1. Extraction Procedure

4.1.1. Hexane ( $\text{CH}_3(\text{CH}_2)_4\text{CH}_3$ ), residue-free, distilled in glass.

4.1.2. Dichloromethane ( $\text{CH}_2\text{Cl}_2$ ), residue-free, distilled in glass.

4.1.3. Sodium sulphate ( $\text{Na}_2\text{SO}_4$ ), anhydrous, extracted with dichloromethane, dried and stored at  $130^\circ\text{C}$ .

4.1.4. Diethyl ether ( $(\text{C}_2\text{H}_5)_2\text{O}$ ), residue free, distilled in glass.

4.1.5. Iso-octane, (2,2,4-trimethyl pentane),  $(\text{CH}_3)_3\text{CCH}_2\text{CH}(\text{CH}_3)_2$ .

4.1.6. Sodium hydroxide ( $\text{NaOH}$ ), concentrated solution.

4.1.7. Phosphoric acid ( $\text{H}_3\text{PO}_4$ ), concentrated.

4.1.8. Glass fibre filter paper, solvent-extracted.

### 4.2. Methylation Procedure

4.2.1. Diazomethane solution in ether, obtained from N,methyl-N,nitrosotoluene-p-sulphonamide using the method of the U.S. Food and Drug Administration Anal. Manual Vol. 1, Sect. 221.14.

4.2.2. Diethyl ether ( $(\text{C}_2\text{H}_5)_2\text{O}$ ), residue free, distilled in glass.

### 4.3. Clean-up Procedure

4.3.1. Florisil, Floridin 60-100 PR stored at  $130^\circ\text{C}$ .

4.3.2. Glass wool, solvent extracted.

4.3.3. As above in 4.1.1, 4.1.2, 4.1.4 and 4.1.5.

### 4.4. Gas Chromatographic Analysis

4.4.1. Methanol ( $\text{CH}_3\text{OH}$ ), distilled in glass.

4.4.2. Hexane ( $\text{CH}_3(\text{CH}_2)_4\text{CH}_3$ ), residue-free, distilled in glass.

#### 4.4.3. Stock Standard Solutions

In a volumetric flask dissolve 0.01 g of each herbicide in methanol and dilute to 100 ml with methanol.

**4.4.4. Working Standard Mixture (1 µg/ml)**

In a volumetric flask dilute 1 ml of each stock standard solution to 100 ml with methanol.

**4.4.5. Methylated Standard Mixture (0.05 µg/ml of methyl esters)**

One ml of the working standard mixture is carefully blown-down to dryness and methylated (procedure 5.2). The methylated standard solution is made up to 20 ml with iso-octane.

**5. Procedure**

**5.1. Extraction Procedure**

- 5.1.1. Mark sample level on the outside of the bottle to determine the water volume which will be measured after the samples are extracted.
- 5.1.2. Add sodium hydroxide solution to make samples basic (pH 12).
- 5.1.3. Add 100 ml dichloromethane to the bottle and place on a rotary extractor.
- 5.1.4. Rotate for 15 minutes. Remove and allow samples to settle.
- 5.1.5. Using a 50 ml pipette attached to a vacuum line remove the lower solvent layer from the bottle.
- 5.1.6. Transfer solvent and carefully discard.
- 5.1.7. Repeat twice more using 50 ml dichloromethane.

**NOTE:** Triazines can be analyzed simultaneously by keeping these dichloromethane extracts for GC analysis.

- 5.1.8. Acidify sample with phosphoric acid to pH 2.
- 5.1.9. Repeat dichloromethane extractions, this time transferring solvent to an Erlenmeyer flask.
- 5.1.10. Add sufficient anhydrous sodium sulphate to absorb any visible water in the flask.
- 5.1.11. Further dry solvent by passing it through a filter funnel containing a glass fibre filter and 10 g sodium sulphate and collect in a 300 ml round bottom flask.
- 5.1.12. Rinse the flask twice with 15 ml aliquots of dichloromethane and pass through the sodium sulphate.
- 5.1.13. Rinse the filter cake with a further 10 ml aliquot of dichloromethane.
- 5.1.14. Place the round bottom flask on a rotary evaporator and evaporate to 5 ml under reduced pressure.
- 5.1.15. Transfer to a 50 ml graduated centrifuge tube and add 2 ml of iso-octane.
- 5.1.16. Reduce the volume to 0.5 ml using a Vortex evaporator. The sample is now ready for methylation.

## 5.2. Methylation Procedure

- 5.2.1. To the dry extract (5.1.16), add 2 ml of diazomethane solution in ether.
- 5.2.2. Allow to stand for one hour minimum.
- 5.2.3. Add 2 ml of iso-octane and warm to 25°C for 20 minutes.
- 5.2.4. Reduce the volume, using a Vortex evaporator until no ether smell remains (0.5 ml). The sample is now ready for Florisil cleanup.

**NOTE:** Extreme care must be taken during the evaporation stages to prevent loss of volatiles.

## 5.3. Clean-up Procedure

- 5.3.1. Rinse column and reservoir with hexane.
- 5.3.2. Plug lower end with 0.5 cm glass wool.
- 5.3.3. Pour sufficient Florisil into beaker to cool and cover with hexane to form a slurry.
- 5.3.4. Pack column with Florisil slurry, to a height of 16 cm, using a Pasteur pipette.
- 5.3.5. Rinse column with 30 ml hexane. Transfer methylated sample to column using a Pasteur pipette.
- 5.3.6. Rinse sample container with a 1 ml aliquot of eluting solvent and transfer to column.
- 5.3.7. Elute with: a) 20 ml of hexane, b) 25 ml of 20% dichloromethane/hexane and c) 50 ml of 7.5% diethyl ether/dichloromethane.
- 5.3.8. Collect fractions in 50 ml centrifuge tubes. Fraction a) is discarded, fraction b) will contain any chlorinated phenolic material and fraction c) will contain 2,4-D type herbicides as their methyl esters.
- 5.3.9. Add 1 ml iso-octane to fraction c) to act as a keeper.
- 5.3.10. Using a stream of dry air, carefully evaporate almost to dryness. Make up to the required volume with iso-octane.

**NOTE:** Extreme care must be taken in all transfer and evaporation stages as considerable losses can occur due to the high volatility of some of the herbicides.

## 5.4. Gas Chromatographic Analysis

### 5.4.1. Operating Conditions:

#### 5.4.1.1. Temperatures:

injector	250°C
detector	300°C
column	90 to 240°C at 3 degrees per minute

#### 5.4.1.2. Gas Flows:

Helium 0.5 ml/min.

5.4.1.3. 50 m, narrow bore, fused silica capillary column with OV101.

- 5.4.2. Allow gas chromatograph to equilibrate at required temperatures and gas flows. Inject methylated standard mixture (4.4.5) to obtain retention times.
- 5.4.3. Repeat 5.4.2. to check reproducibility.
- 5.4.4. Inject 1  $\mu$ l aliquots of sample. Compare retention times of standards to that of peaks in sample. If sample contains any corresponding peaks repeat the injection.
- 5.4.5. Calibrate the instrument by injecting three aliquots of the methylated standard mixture corresponding to different concentrations. Draw calibration curve, plotting peak areas against concentration.

The following herbicides are determined by this method: Dicamba, 2,4-D; Silvex; 2,4,5-T; 2,4-DB; 2,4-DP. MCPA and MCPP may also be analyzed, upon request.

## 6. Calculation and Reporting

Herbicide concentrations are determined from a preconstructed calibration curve. Results are reported in  $\mu$ g/l, of methyl esters.

## 7. Precision and Accuracy

Not available.

## 8. Bibliography

U.S. Environmental Protection Agency. 1976. Analysis of Pesticide Residues in Human and Environmental Samples. Environmental Toxicology Div., Health Effects Laboratory, Research Triangle Park, N.C.

U.S. Department of Health, Education and Welfare. 1965. Guide to the Analysis of Pesticide Residues - Volumes 1 and 2. Public Health Service, Bureau of State Services (Environmental Health), Office of Pesticides, Washington, D.C.

## THE DETERMINATION OF PHOSPHORUS

Phosphorus is a naturally occurring element essential to plant growth and microorganism activity. In waters, soils and sediments phosphorus occurs in the inorganic form as orthophosphate usually in combination with  $H^+$ ,  $Ca^{2+}$ ,  $Fe^{3+}$  or  $Al^{3+}$  and as polyphosphates. In the organic form phosphorus occurs in a variety of compounds which include ADP and ATP in waters. In acidic soils, phosphorus is found primarily in combination with iron and aluminum, whereas in alkaline soils calcium phosphates predominate. Plant available phosphorus occurs almost exclusively as orthophosphate in the soil solution.

Phosphorus is used in all metabolic processes and energy transformations in living cells. Inorganic phosphorus enters the cell and is incorporated into many organic compounds. Upon the death and decomposition of the plant or animal, organic phosphorus compounds are released to the soil or water where they are eventually mineralized into inorganic phosphorus by microbial and enzyme activity.

Phosphorus levels in the environment are increased significantly by man's activities. Major sources of phosphorus occur through wastewater discharges of domestic laundry detergents, commercial laundry detergents, sewage and industrial effluents. Phosphate fertilizers increase soil phosphorus and contaminate ground waters and runoff waters. Large influxes of phosphorus into the water system result in an overabundance of algae and aquatic plants leading to accelerated eutrophication of the water body. Phosphate removal programs at sewage plants can effectively lower phosphate loadings to the environment. Ministry of the Environment guidelines suggest total phosphorus levels of less than 20  $\mu g/l$  to prevent nuisance concentrations of algae.

Weak beta-emitting radionuclides can be used as tracers in biological and medical studies and  $^{32}P$  is an isotope commonly used for such studies. By measuring  $^{32}P$  in water containing algae, the rate of uptake of biologically available phosphate-like material by aquatic plants can be investigated.

### Sample Handling and Preservation

#### Water, Sewage and Industrial Waste

Glass or polystyrene containers are acceptable. Recycled containers may contain phosphate detergent residuals which cannot completely be removed by rinsing. Acid washed containers may activate container walls and result in phosphate adsorption from sample solutions. Linear polyethylene containers should be avoided since dissolved and particulate components of the sample may be lost to the inner walls of the container.

In order to obtain the most reliable results for filtered phosphorus fractions, samples should be filtered in the field at the time of collection and analyzed as soon as possible. Filtered samples from remote areas or unfiltered samples should be refrigerated ( $4^{\circ}C$ ) or frozen to retard biological activity during shipment. Preservation with acid, chloroform or mercurous chloride should be avoided as the phosphorus forms may be altered.

Preservation techniques are not required for total phosphorus; however, the sample may be treated in order to preserve its ammonia content as total phosphorus and total Kjeldahl

nitrogen determinations are often performed on the same aliquot. Freezing or sample acidification with 1 ml concentrated sulphuric acid per liter of sample prevents the loss of ammonia. However, freezing is preferred as it allows total and filtered fractions of phosphorus to be determined on the same sample (assuming filtration at the laboratory rather than in the field).

#### Water for $^{32}\text{P}$ Analysis

Samples of water containing  $^{32}\text{P}$  and algae or other growing plant material are filtered through 0.45  $\mu\text{m}$  pore size Millipore filters. The filters are partially dried in air and placed in a liquid scintillation medium in a polyethylene vial. Standards of known activity are prepared at the same time for use in the determination of the amount of  $^{32}\text{P}$  taken up by the plants. No special preservation techniques are applied but normal safety precautions for handling weakly radioactive substances must be observed.

#### Vegetation

Fresh samples may be refrigerated at 3 - 5°C until further treatment. If requested, samples are washed with Alconox-EDTA solution and rinsed with distilled water. Samples are force dried in a forced air oven, ground in a Wiley Mill to pass a 60 mesh sieve and stored in a sealed glass jar.

#### Soils and Sediments

Samples should be collected in glass jars, air dried and ground to less than 2 mm. At least 1 gram of sample should be taken.

#### **Selection of Method**

Method A is used on surface water, sewage and industrial waste samples for the determination of filtered reactive phosphorus. Samples are filtered and orthophosphate is determined, on the filtrate, colorimetrically after the formation of a reduced phospho-antimonyl-molybdate-complex using ascorbic acid as the reducing agent. Method B uses the same colorimetric finish, but samples are first autoclaved for 45 minutes in a sulphuric acid-persulphate media. This method is used for total filtered phosphorus determinations on surface waters. Method C determines total phosphorus on surface water, sewage and industrial wastes after sample digestion in a sulphuric acid-mercuric oxide-potassium sulphate media. The colorimetric finish is the same as that described in Method A.

Method D, liquid scintillation counting, is used for the measurement of  $^{32}\text{P}$  in water samples. This method is a sensitive technique for the analysis of weak  $\beta$ -emitting radionuclides used as tracers in numerous biological and medical applications. This method is uniquely suited to the routine determination of  $^{32}\text{P}$  activity in solid matrices deposited on filter media.

Method E, is an X-ray fluorescence method used for total phosphorus determinations on vegetation samples. A brief summary of the XRF method is provided, however, the detailed description of the method is given in THE DETERMINATION OF TRACE.

Method F is an acid dissolution-automated molybdenum blue colorimetric procedure used for total phosphorus determinations on soil and sediment samples. The reducing agent used in the colorimetric step is stannous chloride.



## FILTERED REACTIVE PHOSPHORUS

### Filtered - Automated Ascorbic Acid Colorimetry Method A

#### SUMMARY

<b>Matrix.</b>	This method is used on surface water samples.
<b>Substance determined.</b>	Filtered, reactive phosphorus.
<b>Interpretation of results.</b>	Results are reported in mg/l P.
<b>Principle of method.</b>	A sample is filtered and orthophosphate in the filtrate is determined by the formation of a phospho-antimonyl-molybdate complex using ascorbic acid as the reducing agent. Color is measured using an AutoAnalyzer II system at 880 nm.
<b>Time required for analysis.</b>	Approximately 200 samples may be run each day.
<b>Range of application.</b>	0.002 - 0.100 mg/l P.
<b>Standard deviation.</b>	The average standard deviation based on within run duplicates is 0.0014. For a more detailed breakdown see Precision and Accuracy, Section 7.
<b>Accuracy.</b>	The average recoveries for two quality control solutions were 103.4% with relative standard deviations of 1.9% and 4.9%. These data were based on 43 operating days.
<b>Detection criteria.</b>	0.0024 mg/l phosphorus.
<b>Interferences and shortcomings.</b>	Arsenate (As V) above 10 $\mu$ g/l interferes by forming a heteropoly blue complex which adds to the apparent P concentration. Ferric ion and silicates above 50 mg/l may interfere.
<b>Minimum volume of sample.</b>	75 ml.
<b>Preservation and sample container.</b>	Glass or new polystyrene containers are required. Samples may be filtered in the field. Samples should be refrigerated or frozen.
<b>Safety considerations.</b>	Exercise caution when handling concentrated acids.

## FILTERED REACTIVE PHOSPHORUS

### Filtered - Automated Ascorbic Acid Colorimetry Method A

#### 1. Introduction

Samples are filtered in the field or in the laboratory. Orthophosphate is determined by the formation of a reduced phospho-antimonymolybdate complex using ascorbic acid as the reducing agent.

#### 2. Interferences and Shortcomings

Arsenic as arsenate in concentrations above 10  $\mu\text{g/l}$  causes positive interference by the formation of a heteropoly blue complex which adds to the apparent concentration. Ferric ion and silicates above 50 mg/l also interfere. It is possible that some organic and particulate forms of phosphorus will react similarly to dissolved orthophosphate under the analytical test conditions. Humates and tannates may also interfere.

#### 3. Apparatus

- 3.1. Filtration apparatus, Figure 1.
- 3.2. Filter paper, glass fibre, 4.25 cm, Reeve Angel 934 AH.
- 3.3. AutoAnalyzer system including the following:
  - 3.3.1. automated sampler.
  - 3.3.2. proportioning pump.
  - 3.3.3. AAII colorimeter equipped with 880 nm filters and 199-B02104 phototubes plus a 5 cm flow cell.
  - 3.3.4. voltage regulator.
  - 3.3.5. chart recorder.
- 3.4. Pump tubing and assorted manifold glassware as in Figure 2.
- 3.5. Culture tubes, 18 x 150 mm and tube racks.
- 3.6. Dilution tubes, 50 ml.

#### 4. Reagents

- 4.1. Potassium dihydrogen phosphate ( $\text{KH}_2\text{PO}_4$ ), anhydrous reagent grade powder.
- 4.2. Ammonium molybdate tetrahydrate ( $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}$ ), reagent grade crystals.

- 4.3. Sulphuric acid ( $\text{H}_2\text{SO}_4$ ), concentrated reagent grade.
- 4.4. Antimony potassium tartrate semihydrate ( $\text{K}(\text{SbO})\text{C}_2\text{H}_4\text{O}_6 \cdot \frac{1}{2}\text{H}_2\text{O}$ ), reagent grade crystals.
- 4.5. Ascorbic acid ( $\text{C}_6\text{H}_8\text{O}_6$ ), reagent grade crystals.

**4.6. Stock Sulphuric Acid Solution (20% v/v)**

In a 4 liter Pyrex beaker, carefully add 800 ml concentrated sulphuric acid to approximately 3 liters of constantly stirred distilled, deionized water. Allow resulting solution to cool prior to diluting to a final volume of 4 liters.

**NOTE:** Eye protection and gloves must be worn and extreme caution must be exercised during the preparation of this solution.

**4.7. Working Molybdate Reagent**

Dissolve 48 g ammonium molybdate tetrahydrate in 2400 ml stock sulphuric acid solution (20% v/v) with constant stirring. Cool, and add 1.00 g antimony potassium tartrate. Dissolve and dilute to 4 liters with distilled, deionized water.

**NOTE:** Wear eye protection and gloves when adding the sulphuric acid solution.

**4.8. Working Ascorbic Acid Reagent**

Dissolve 20 g ascorbic acid in distilled, deionized water and dilute to 1 liter. Although this solution is sufficiently stable for 3 days, daily preparation is recommended.

**4.9. Quality Control Stock Solution (200 mg/l P)**

Dissolve 0.8780 anhydrous potassium dihydrogen phosphate in distilled, deionized water. Dilute to 1 liter in a volumetric flask.

**4.10. Intermediate Quality Control Solution (5 mg/l P)**

Dilute 25 ml of quality control stock solution to 1 liter with distilled, deionized water in a volumetric flask.

**4.11. Quality Control Working Solutions**

QC-A - Dilute 15 ml intermediate quality control solution to 1 liter in a volumetric flask. This gives a phosphorus concentration of .075 mg/l (75% of full scale).

QC-B - Dilute 5 ml intermediate quality control solution to 1 liter in a volumetric flask. This gives a phosphorus concentration of .025 mg/l (25% of full scale).

**4.12. Calibration Stock Solution (200 mg/l P)**

Dissolve 1.756 g anhydrous potassium dihydrogen phosphate in distilled, deionized water. Dilute to 2 liters in a volumetric flask.

**4.13. Intermediate Calibration Solution (4 mg/l P)**

Dilute 20 ml concentrated stock standard solution to 1 liter with distilled, deionized water in a volumetric flask.

#### 4.14. Daily Calibration Standards

Prepare a high standard (H) at 80% of the working range (0.080 mg/l P) by diluting 20 ml intermediate calibration solution to 1 liter with distilled, deionized water in a volumetric flask. Prepare weekly.

Prepare a low standard (L) at 20% of range (0.020 mg/l P) by diluting 5.0 ml intermediate calibration solution to 1 liter with distilled, deionized water in a volumetric flask. Prepare weekly.

### 5. Procedure

- 5.1. Collect the samples and group them according to the lab numbers shown on the bench sheet.
- 5.2. Prepare the filtration apparatus as shown in Figure 1.
- 5.3. Using only forceps, place the appropriate glass fibre filter in the funnel.
- 5.4. Fit a clean 19 x 150 mm culture tube onto the adaptor, being careful not to touch the rim or inside glass surface of the tube or the rubber adaptor.
- 5.5. Shake the sample vigorously and promptly vacuum filter two 15 ml portions, discarding the filtrate each time.
- 5.6. Collect the third portion of the filtrate for analysis. Remove the contaminated filter paper with the forceps.
- 5.7. Place the culture tube containing the filtered sample into a test tube rack in such a position that it may easily be correlated with the sample number on the bench sheet. Write the appropriate sample number on the first and last sample tube in each row.
- 5.8. Set the AutoAnalyzer into operation using cleaning, set-up and checking procedures appropriate to the manifold illustrated in Figure 2.
- 5.9. When loading the samples into the AutoAnalyzer sampler module, ensure that sample order conforms to the bench sheet.
- 5.10. Each run of samples will include all of the following units:

Set of calibration standards: H, L  
DDW blank (day's supply): Bl  
Quality Control samples: QC-A, QC-B  
Long term blank: LTBl

The basic sample loading sequence is: 10 samples, Bl, 10 samples, L, H, Bl.

- 5.11. Calibrate the AutoAnalyzer system using calibration standards. Record the standard calibration setting, and check that it has not changed unduly.
- 5.12. Confirm the calibration by analyzing the quality control solutions. Record these values. For each analytical range, calculate totals and differences, e.g., QC-A plus QC-B and QC-A minus QC-B. Check to ensure that the calculated values conform to limits based on past data.
- 5.13. Monitor calibration standards (in-run sensitivity checks) throughout the run to determine if within-run corrections are required.

5.14. Read sample peak heights, and convert to concentration values.

## 6. Calculation and Method of Reporting

If required multiply the reading by the dilution factor

$$\text{dilution factor} = \frac{\text{diluted volume}}{\text{aliquot volume}}$$

and record the result in the answer space opposite each sample number of the bench sheet. Report the results to the nearest 0.001 if feasible.

## 7. Precision and Accuracy

Precision - Duplicate Analyses of Routine Samples

Samples Concentrations mg/l as P	Standard Deviation mg/l as P
<0.020	0.0018
0.020 - 0.050	0.0021
0.050 - 0.100	0.0035
Average	0.0020

Accuracy - Recovery

QC Concentration mg/l as P	Average Recovery mg/l as P	Standard Deviation mg/l as P
0.075	0.0753	0.0014
0.025	0.0266	0.0012

## 8. Bibliography

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- 8.4. Murphy, J. and Riley, J. P. (1962). A modified single solution method for the determination of phosphate in natural water. *Analytica Chimica Acta* 27: 31-36.

## FILTERED REACTIVE PHOSPHORUS

### Filtered - Automated Ascorbic Acid Colorimetry Method A - Variation 1

#### SUMMARY

<b>Matrix.</b>	This method is used on domestic water, sewage and industrial waste samples.
<b>Substance determined.</b>	Filtered, reactive phosphorus.
<b>Interpretation of results.</b>	Results are reported in mg/l P.
<b>Principle of method.</b>	A sample is filtered and orthophosphate in the filtrate is determined by the formation of a phospho-antimonyl-molybdate complex using ascorbic acid as the reducing agent. Color is measured using an AutoAnalyzer II system at 880 nm.
<b>Time required for analysis.</b>	Approximately 200 samples may be run each day.
<b>Range of application.</b>	Dual ranges 0.04 - 4.00 and 4.0 - 10.0 mg/l P.
<b>Standard deviation.</b>	The average standard deviations based on within run duplicates are 0.049 for the 0.04 to 4.0 mg/l range and 0.107 for the 4.0 - 10.0 mg/l range. For a more detailed breakdown see Precision and Accuracy, Section 7.
<b>Accuracy.</b>	Recoveries for four quality control solutions ranged from 94% to 100.5%. Relative standard deviations ranged from 0.87% to 3.2%.
<b>Detection criteria.</b>	0.057 mg/l P.
<b>Interferences and shortcomings.</b>	Arsenate (As V) above 100 $\mu$ g/l interferes by forming a heteropoly blue complex which adds to the apparent P concentration. Ferric ion and silicates above 500 mg/l may interfere. Humates and tannates may interfere.
<b>Minimum volume of sample.</b>	75 ml.
<b>Preservation and sample container.</b>	Glass or new polystyrene containers are required. Samples may be filtered in the field. Samples should be refrigerated or frozen.
<b>Safety considerations.</b>	Exercise caution when handling concentrated acids.

## FILTERED REACTIVE PHOSPHORUS

### Filtered - Automated Ascorbic Acid Colorimetry Method A

#### 1. Introduction

Samples are filtered in the field or in the laboratory. Orthophosphate is determined by the formation of a reduced phospho-antimonymolybdate complex using ascorbic acid as the reducing agent.

#### 2. Interferences and Shortcomings

Arsenic as arsenate in concentrations above 100  $\mu\text{g/l}$  causes positive interference by the formation of a heteropoly blue complex which adds to the apparent concentration. Ferric ion and silicates above 500  $\text{mg/l}$  also interfere. It is possible that some organic and particulate forms of phosphorus will react similarly to dissolved orthophosphate under the analytical test conditions. Humates and tannates may also interfere.

#### 3. Apparatus

- 3.1. Technicon continuous filter module as in Figures 3 and 4.
- 3.2. Filter paper, glass fibre, Schleicher and Schuell grade 410.
- 3.3. AutoAnalyzer system including the following:
  - 3.3.1. automated sampler.
  - 3.3.2. proportioning pump.
  - 3.3.3. AAI colorimeter equipped with 880 nm filters and 199-B02104 phototubes plus a 5 cm flow cell.
  - 3.3.4. voltage regulator.
  - 3.3.5. chart recorder.
- 3.4. Pump tubing and assorted manifold glassware as in Figure 5.
- 3.5. Culture tubes, 18 x 150 mm and tube racks.
- 3.6. Dilution tubes, 50 ml.

#### 4. Reagents

- 4.1. Potassium dihydrogen phosphate ( $\text{KH}_2\text{PO}_4$ ), anhydrous reagent grade powder.
- 4.2. Ammonium molybdate tetrahydrate ( $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}$ ), reagent grade crystals.

- 4.3. Sulphuric acid ( $\text{H}_2\text{SO}_4$ ), concentrated reagent grade.
- 4.4. Antimony potassium tartrate semihydrate ( $\text{K}(\text{SbO})\text{C}_2\text{H}_4\text{O}_6 \cdot \frac{1}{2}\text{H}_2\text{O}$ ), reagent grade crystals.
- 4.5. Ascorbic acid ( $\text{C}_6\text{H}_8\text{O}_6$ ), reagent grade crystals.

**4.6. Stock Sulphuric Acid Solution (20% v/v)**

In a 4 liter Pyrex beaker, carefully add 800 ml concentrated sulphuric acid to approximately 3 liters of constantly stirred distilled, deionized water. Allow resulting solution to cool prior to diluting to a final volume of 4 liters.

**NOTE:** Eye protection and gloves must be worn and extreme caution must be exercised during the preparation of this solution.

**4.7. Working Molybdate Reagent**

Dissolve 48 g ammonium molybdate tetrahydrate in 2400 ml stock sulphuric acid solution (20% v/v) with constant stirring. Cool, and add 1.00 g antimony potassium tartrate. Dissolve and dilute to 4 liters with distilled, deionized water.

**NOTE:** Wear eye protection and gloves when adding the sulphuric acid solution.

**4.8. Working Ascorbic Acid Reagent**

Dissolve 20 g ascorbic acid in distilled, deionized water and dilute to 1 liter. Although this solution is sufficiently stable for 3 days, daily preparation is recommended.

**4.9. Quality Control for Sewage and Industrial Waste (0.04 - 10.00 mg/l P)**

If ammonia, nitrate and nitrite are also to be determined combined quality control solutions may be prepared. See the appropriate method write-up for solution concentration.

**4.10. Quality Control Stock Solution (280 mg/l P)**

Dissolve 1.2302 g potassium di-hydrogen phosphate in distilled, deionized water and dilute to 1 liter.

**4.11. Quality Control Working Solutions**

QC-A - Dilute 50 ml quality control stock solution to 2 liters with distilled, deionized water to give a phosphorus concentration of 7.0 mg/l P (70% of full scale).

QC-B - Dilute 25 ml quality control stock solution to 2 liters with distilled, deionized water to give a phosphorus concentration of 3.5 mg/l P (35% of full scale).

QC-C - Dilute 5 ml quality control stock solution to 2 liters with distilled, deionized water to give a phosphorus concentration of 0.70 mg/l P (7% of full scale).

**4.12. Calibration Stock Solutions (1600 mg/l P)**

Dissolve 7.030 g potassium di-hydrogen phosphate in 500 ml distilled, deionized water and dilute to 1 liter.



#### 4.13 Intermediate Calibration Solution (160 mg/l P)

Dilute 200 ml calibration stock solution to 2 liters with distilled, deionized water and store in the refrigerator.

#### 4.14. Daily Calibration Standards

Prepare a high (H) standard by pipetting 50 ml calibration intermediate solution to 1 liter with distilled, deionized water. This gives a phosphorus concentration of 8.0 mg/l. Prepare weekly.

Prepare a low (L) standard by pipetting 20 ml calibration intermediate solution to 1 liter with distilled, deionized water. This gives a phosphorus concentration of 3.2 mg/l. Prepare weekly.

### 5. Procedure

- 5.1. Collect the samples and group them according to the bench sheet.
- 5.2. Prepare the continuous filtration apparatus as shown in Figures 3 and 4.
- 5.3. Place a culture tube containing the sample into a test tube rack in such a position that it may easily be correlated with the sample number on the bench sheet. Write the appropriate sample number on the first and last sample tube in each row.
- 5.4. Set the AutoAnalyzer into operation using cleaning, set-up and checking procedures appropriate to the manifold illustrated in Figures 3, 4 and 5.
- 5.5. When loading the samples into the AutoAnalyzer sampler module, ensure that sample order conforms to the bench sheet.
- 5.6. Each run of samples will include all of the following units:

Set of calibration standards: H, L  
DDW blank (day's supply): Bl  
Quality Control samples: QC-A, QC-B, QC-C  
Long term blank: LTBl

The basic sample loading sequence is: 10 samples, Bl, 10 samples, L, H, Bl.

- 5.7. Calibrate the AutoAnalyzer system using calibration standards. Record the standard calibration setting, and check to ensure that it has not changed unduly.
- 5.8. Confirm the calibration by analyzing the quality control solutions. Record these values. For each analytical range, calculate totals and differences, e.g., QC-A plus QC-B and QC-A minus QC-B. Check to ensure that the calculated values conform to limits based on past data. QC-A and QC-B are used to check the calibration of the 0 - 10 mg/l P range while QC-B and QC-C are used for the 0 - 4.00 mg/l P range.
- 5.9. Monitor calibration standards (in-run sensitivity checks) throughout the run to determine if within-run corrections are required.
- 5.10. Read sample peak heights, and convert to concentration values.

## 6. Calculation and Method of Reporting

If required multiply the reading by the dilution factor

$$\text{dilution factor} = \frac{\text{diluted volume}}{\text{aliquot volume}}$$

Record the result in the answer space opposite each sample number on the bench sheet. Report the results to the nearest 0.01 if feasible.

## 7. Precision and Accuracy

Precision - Within-run standard deviations based on duplicate samples are as follows:

Range mg/l as P	Sample Concentrations mg/l as P	Standard Deviation mg/l as P
0 - 4.00	<0.8	0.0348
	0.80 - 2.00	0.0684
	2.00 - 4.00	0.0414
	Average	0.0493
0 - 10.0	<2.00	-
	2.00 - 5.00	0.141
	5.00 - 10.0	0.075
	Average	0.107

Accuracy - Recovery

Range mg/l as P	QC Concentrations mg/l as P	Average Recovery mg/l P	Standard Deviation mg/l P
0 - 4.00	3.50	3.517	0.0304
	0.70	0.657	0.0227
0 - 10.0	7.0	7.03	0.065
	3.5	3.51	0.035

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## FILTERED TOTAL PHOSPHORUS

### Autoclave - Automated Ascorbic Acid Colorimetry Method B

#### SUMMARY

<b>Matrix.</b>	This method is used for filtered total phosphorus determinations on surface waters.
<b>Substance determined.</b>	Filtered total phosphorus; (total soluble P).
<b>Interpretation of results.</b>	Results are reported in $\mu\text{g/l P}$ .
<b>Principle of method.</b>	Samples are autoclaved for 45 minutes in sulphuric acid-per-sulphate media at $121^{\circ}\text{C}$ and presented to AAI system in the sample container used for collection. Orthophosphate in the digestate is determined by the formation of reduced phospho-antimonyl-molybdate complex using ascorbic acid as the reducing agent.
<b>Time required for analysis.</b>	Approximately 150 samples can be analyzed per day.
<b>Range of application.</b>	Dual Ranges: 0.7 - 20.0 and 20.0 to 200 $\mu\text{g/l P}$ .
<b>Standard deviation.</b>	The average standard deviations based on within run duplicates are 0.437 for the 0.7 to 20.0 $\mu\text{g/l}$ range and 1.26 for the 20.0 to 200 $\mu\text{g/l}$ range.
<b>Accuracy.</b>	Recoveries for four quality control solutions ranged from 98.8% to 102.0%. Standard deviations for these solutions ranged from 0.211 $\mu\text{g/l}$ to 1.86 $\mu\text{g/l}$ .
<b>Detection criteria.</b>	0.7 $\mu\text{g/l}$ as Phosphorus.
<b>Interferences and shortcomings.</b>	Arsenate above 25 $\mu\text{g/l}$ , ferric iron and silicates above 50 $\text{mg/l}$ interfere with the colorimetric determination of phosphorus. The digestion conditions may not be vigorous enough to release organically bound phosphorus from particulate matter.
<b>Minimum sample size.</b>	1 tube filled above the 40 ml mark.
<b>Preservation and sample container.</b>	Submit samples in calibrated 25 x 150 mm Pyrex or Kimax tubes fitted with Teflon lined Bakelite caps. Samples should be filtered in the field as soon after collection as possible using Sartorius 0.45 $\mu\text{m}$ cellulose acetate filters with no spacers.
<b>Safety considerations.</b>	Exercise caution when handling concentrated acids.

## **FILTERED TOTAL PHOSPHORUS**

### **Autoclave - Automated Ascorbic Acid Colorimetry Method B**

#### **1. Introduction**

Filtered samples are aspirated down to 35 ml and autoclaved in the sample tubes for 45 minutes in 5 ml of a sulphuric acid-persulphate media at 121°C. Acid hydrolyzable phosphate and some soluble organic phosphate compounds present in the sample are converted to orthophosphate. The tubes are then placed in an AAI AutoAnalyzer system and orthophosphate is determined by the formation of the reduced phosphoantimonymolybdate complex using ascorbic acid as the reducing agent.

#### **2. Interferences and Shortcomings**

Interferences include arsenate above 25 µg/l As and ferric iron and silicates above 50 mg/l. The digestion step may not be severe enough to release organically bound phosphorus from particulate matter.

#### **3. Apparatus**

3.1. Filtration apparatus for 47 mm membrane filters.

3.2. Filters, Sartorius, 0.45 µm, cellulose acetate, no spacers..

3.3. Auto Analyzer AAI system including the following:

3.3.1. Automated sampler.

3.3.2. Proportioning pump.

3.3.3. AA-II colorimeter equipped with 880 nm filters and 199-B02104 phototubes and 5 cm flow cells.

3.3.4. Voltage regulator.

3.3.5. Dual pen chart recorder.

3.4. Pump tubing and assorted glassware as in Figure 6.

3.5. Pyrex or Kimax tubes, 25 x 150 mm fitted with Teflon lined caps and tube racks. Tubes should be marked at the 35 ml and 40 ml levels.

3.6. Dilution tubes, 50 ml.

3.7. Autoclave.

3.8. Oxford pipettor.

#### 4. Reagents

- 4.1. Potassium dihydrogen phosphate ( $\text{KH}_2\text{PO}_4$ ), anhydrous reagent grade powder.
- 4.2. Ammonium molybdate tetrahydrate ( $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}$ ), reagent grade crystals.
- 4.3. Sulphuric acid ( $\text{H}_2\text{SO}_4$ ), concentrated reagent grade.
- 4.4. Antimony potassium tartrate semihydrate ( $\text{K}(\text{SbO})\text{C}_2\text{H}_3\text{O}_6 \cdot \frac{1}{2}\text{H}_2\text{O}$ ), reagent grade crystals.
- 4.5. Ascorbic acid ( $\text{C}_6\text{H}_8\text{O}_6$ ), reagent grade crystals.
- 4.6. Potassium persulphate ( $\text{K}_2\text{S}_2\text{O}_8$ ), reagent grade crystals.
- 4.7. Sodium pyrophosphate ( $\text{Na}_4\text{P}_2\text{O}_7$ ), anhydrous, reagent grade crystals.

##### 4.8. Stock Molybdate Solution

Dissolve 48 g ammonium molybdate tetrahydrate in 3 liters distilled, deionized water. Add 355 mL concentrated sulphuric acid. Allow the solution to cool and then dissolve 1.0 g potassium antimony tartrate semihydrate in the solution. Dilute to 4 liters.

**NOTE:** Wear gloves and safety glasses when preparing this solution.

##### 4.9. Ascorbic Acid Solution (1%)

Dissolve 4.0 g ascorbic acid in distilled, deionized water and dilute to 400 ml. Prepare fresh daily.

##### 4.10. Wash Water

Dilute 25 ml concentrated sulphuric acid to 4 liters with distilled, deionized water. Prepare fresh daily.

##### 4.11. Digestion Acid

To 500 ml of distilled, deionized water add 25 g of potassium persulphate and 20 ml of concentrated sulphuric acid. Prepare fresh daily and add to samples just before autoclaving.

**NOTE:** Wear gloves and safety glasses when preparing this solution.

##### 4.12. Quality Control Stock Solution (100 mg/l P)

Dissolve 0.8788 g of potassium dihydrogen phosphate in distilled, deionized water and dilute to 2 liter in a volumetric flask. Store in a refrigerator.

##### 4.13. Quality Control Intermediate Solution for the 20.0-200 $\mu\text{g/l}$ P Range (10 mg/l P)

In a volumetric flask dilute 200 ml of the 100 mg/l P stock to 2 liters with distilled, deionized water. Store this solution in specially prepared ampules.

#### **4.14. Daily Quality Control Solutions for the 20.0–200 µg/l P Range**

QC-A: In a volumetric flask, dilute 30 ml of the intermediate stock (10 mg/l P) to 2 liters with fresh wash water. This gives a phosphorus concentration of 150 µg/l P.

QC-B: In a volumetric flask, dilute 10 ml of the intermediate stock (10 mg/l P) to 2 liters with fresh wash water. This gives a phosphorus concentration of 50 µg/l P.

#### **4.15. Intermediate Quality Control Solution for the 0–20.0 mg/l P Range (1 mg/l P)**

In a volumetric flask dilute 20 ml of the 100 mg/l P stock to 2 liters with distilled, deionized water. Store this solution in specially prepared ampules.

#### **4.16. Daily Quality Control Solutions for the 0–20.0 µg/l P Range)**

QC-C: In a volumetric flask, dilute 30 ml of the intermediate stock (1 mg/l P) to 2 liters with fresh wash water. This gives a phosphorus concentration of 15 µg/l P after dilution. See step 5.2.3.

QC-D: In a volumetric flask, dilute 10 ml of the intermediate stock (1 mg/l P) to 2 liters with fresh wash water. This gives a phosphorus concentration of 5 µg/l P after dilution. See step 5.2.3.

Long Term Blank: In a 2 liter flask, store fresh wash water from the same batch that was used to make the daily QC solutions.

#### **4.17. Calibration Stock Solution (200 mg/l P)**

Dissolve 0.8788 g potassium dihydrogen phosphate in distilled, deionized water and dilute to 1 liter. Store in a refrigerator.

#### **4.18. Intermediate Calibration stock for the 20.0 to 200 µg/l P Range (5 mg/l P)**

In a volumetric flask dilute 25 ml of the 200 mg/l P stock to 1 liter with distilled, deionized water. Store in a refrigerator.

#### **4.19. Intermediate Calibration Solution for the 0 – 20.0 µg/l P Range (1 mg/l)**

In a volumetric flask, dilute 5 ml of the 200 mg/l P stock to 1 liter with distilled, deionized water. Store in a refrigerator.

#### **4.20. Daily Calibration Standards**

High: In a volumetric flask, dilute 25 ml of the 5 mg/l P intermediate stock to 1 liter with fresh wash water. Since this standard is not diluted it will read 143 µg/l P. Prepare weekly.

Low: In a volumetric flask, dilute 10 ml of the 1 mg/l P intermediate stock to 1 liter with fresh wash water. Since this standard is not diluted it will read 11.4 µg/l. Prepare weekly.

#### **4.21. Recovery Stock Solution (140 mg/l P)**

Dissolve 0.60093 g of anhydrous sodium pyrophosphate in distilled, deionized water and dilute to 1 liter. Store in a refrigerator.

#### **4.22. Intermediate Recovery Solution (1.40 mg/l P)**

In a volumetric flask, dilute 10 ml of the 140 mg/l P stock to 1 liter with distilled, deionized water.

#### 4.23. Daily Recovery Standards

- R<sub>1</sub>: In a volumetric flask, dilute 100 ml of the 1.40 mg/l P intermediate solution to 1 liter with distilled, deionized water. This gives a phosphorus concentration of 140 µg/l P. Prepare weekly.
- R<sub>2</sub>: In a volumetric flask, dilute 50 mL of the 1.40 mg/l P intermediate solution to 1 liter with distilled, deionized water. This gives a phosphorus concentration of 70 µg/l P. Prepare weekly.
- R<sub>3</sub>: In a volumetric flask, dilute 10 ml of the 1.40 mg/l P intermediate solution to 1 liter with distilled, deionized water. This gives a phosphorus concentration of 14 µg/l P. Prepare weekly.
- R<sub>4</sub>: In a volumetric flask, dilute 5 ml of the 1.40 mg/l P intermediate solution to 1 liter with distilled, deionized water. This gives a phosphorus concentration of 7 µg/l P. Prepare weekly.

### 5. Procedure

#### 5.1 Sample Preparation

- 5.1.1. Collect samples and group them according to the bench sheet. Place the samples in numerical order in racks leaving a space between each sample.
- 5.1.2. Mix the samples well and aspirate to the 35 ml mark on the tubes.
- 5.1.3. Using an Oxford pipettor, add 5 ml of freshly prepared digestion acid. Recap tightly and mix thoroughly.
- 5.1.4. Repeat steps 5.1.1. - 5.1.3. for four duplicate samples and two aliquots of each of the daily recovery solutions.
- 5.1.5. Prepare three digestion acid blanks by pipetting 5 ml of digestion acid into an empty tube.
- 5.1.6. Dilute the three digestion acid blanks to 40 ml with distilled, deionized water mix thoroughly.
- 5.1.7. Prepare three filter blanks by filtering distilled, deionized water through Sartorius CA 0.45 µm filters and collecting the filtrate in samples tubes. Follow steps 5.1.2. and 5.1.3.
- 5.1.8. Autoclave the samples at 121°C for 45 minutes, following the operating instructions carefully. When the cycle is finished, cool samples and then check to see if there has been any significant loss of liquid in any of the tubes. Discard any tubes which show a significant volume loss (i.e. more than 3 ml).

#### 5.2. Phosphorus Determination

- 5.2.1. Set the AutoAnalyzer into operation using cleaning, set-up and checking procedures appropriate to the manifold illustrated in Figure 6.
- 5.2.2. When loading the samples into the AutoAnalyzer sampling module, ensure that the sample order conforms to the bench sheet.



- 5.2.3. Prepare the daily QC standards and long term blank by pouring the solutions into labelled tubes, aspirating to 35 ml and diluting to 40 ml with wash water. Mix thoroughly.
- 5.2.4. Each run of samples will include all of the following units:  
 Set of calibration standards: H, L  
 Wash water blank: Bl  
 Quality Control Standards: QC-A, QC-B, QC-C, QC-D  
 Long term blank: LTB  
 Recovery solutions: R<sub>1</sub>, R<sub>2</sub>, R<sub>3</sub>, R<sub>4</sub>  
 Digested blank: DB  
 Filter Digested Blank: FDB  
 The basic sample loading sequence is:  
 10 samples, Bl, 10 samples, L, H, Bl.
- 5.2.5. Calibrate the AutoAnalyzer unit using the working calibration standards. Record the standard calibration setting, and check to see that it has not changed greatly from the previous day.
- 5.2.6. Confirm the calibration by analyzing the quality control solutions. Record these values. For each analytical range calculate totals and differences, e.g. QC-A plus QC-B and QC-A minus QC-B. Check to ensure that the calculated values conform to limits based on past data.

Summary:

Solution	Digested Yes/No	Acceptable Reading µg/l P
H	No	143
L	No	11.4
Bl	No	0
QC-A	No	150
QC-B	No	50
QC-C	No	15
QC-D	No	5
LTB	No	<1
R <sub>1</sub>	Yes	140
R <sub>2</sub>	Yes	70
R <sub>3</sub>	Yes	14
R <sub>4</sub>	Yes	7
DB	Yes	<1
FDB	Yes	<1

- 5.2.7. Monitor calibration standards (in-run sensitivity checks) throughout the run to determine if within-run corrections are required.
- 5.2.8. Read sample peak heights and convert to concentration values.
- 5.2.9. Any sample readings which are too high for the scale must be diluted with fresh WASH WATER.

## 6. Calculation and Reporting

If required, multiply the reading by the following dilution factor:

$$\text{dilution factor} = \frac{\text{diluted volume}}{\text{aliquot volume}}$$

Record the result in the answer space opposite each sample number on the bench sheet. Report results for the low range to the nearest 0.1  $\mu\text{g/l P}$  and to the nearest 1  $\mu\text{g/l P}$  for the high range.

## 7. Precision and Accuracy

### Precision

Within-run standard deviations based on duplicate samples are as follows:

Range $\mu\text{g/l as P}$	Sample Concentrations $\mu\text{g/l as P}$	Standard Deviations $\mu\text{g/l as P}$
0 - 20.0	<4.0	0.431
	4.0 - 10.0	0.314
	10.0 - 20.0	0.583
	Average	0.437
20.0- 200	<40	-
	40 - 100	1.41
	100 - 200	-
	Average	1.26

### Accuracy - Recovery

Range $\mu\text{g/l P}$	QC Concentrations $\mu\text{g/l P}$	Average Recovery $\mu\text{g/l P}$	Standard Deviation $\mu\text{g/l P}$
0 - 20.0	15.0	15.31	0.513
	5.0	5.09	0.211
20.0 - 200	150	150.9	1.86
	50	49.4	1.09

Range $\mu\text{g/l P}$	Recovery Concentrations $\mu\text{g/l P}$	Average Recovery $\mu\text{g/l P}$	Standard Deviation $\mu\text{g/l P}$
0 - 20.0	14.0	14.30	0.777
	7.0	7.05	0.364
20.0 - 200	140	140.3	1.51
	70	67.9	2.06

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- 8.1. American Public Health Association, American Water Works Association and Water Pollution Control Federation (1975). Standard Methods for the Examination of Water and Wastewater, 14th ed. APHA, Washington, D.C. 624.
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## TOTAL PHOSPHORUS

### Acid Digestion - Automated Ascorbic Acid Colorimetry Method C

#### SUMMARY

Matrix.	This method is used for total phosphorus determinations on surface waters.
Substance determined.	Total phosphorus, P.
Interpretation of results.	Results are reported in mg/l P.
Principle of method.	Samples are digested in sulphuric acid-mercuric oxide-potassium sulphate media using 2 block digesters maintained at 200°C and 360°C. The orthophosphate content in the digestate is determined by the formation of a reduced phospho-antimonylmolybdate complex using ascorbic acid as the reducing agent. pH is controlled throughout.
Time required for analysis.	Samples are digested in batches of 40. Approximately 150 samples can be analyzed per day.
Range of application.	.004 - .200 mg/l for surface waters.
Standard deviation.	For surface waters the average standard deviation is 0.0029. More detailed information is provided in Section 7, Precision and Accuracy.
Accuracy.	Recoveries for two Quality Control solutions were 100.6% and 100.8%. Relative standard deviations were 0.88% to 1.8%.
Detection criteria.	0.0036 mg/l phosphorus.
Interferences and shortcomings.	Arsenate above 25 µg/l, ferric iron above 50 mg/l and silicate above 50 mg/l interfere.
Minimum volume of sample.	75 ml.
Sample container and preservation.	Samples should be submitted in glass or polystyrene containers.
Safety considerations.	Exercise caution when handling concentrated acids.

## TOTAL PHOSPHORUS

### Acid Digestion - Automated Ascorbic Acid Colorimetry Method C

#### 1. Introduction

Samples are digested in sulphuric acid-mercuric oxide-potassium sulphate media using 2 block digesters maintained at 200°C and 360°C. Using an AAI system, within-run pH control is obtained and orthophosphate is measured by the formation of a reduced phospho-antimonymolybdate complex using ascorbic acid as the reducing agent.

#### 2. Interferences and Shortcomings

Interferences include arsenate above 25 µg/l, ferric iron above 50 mg/l and silicate above 50 mg/l.

#### 3. Apparatus

3.1. Auto Analyzer AAI system with the following modules:

- 3.1.1. 2 Block digestors, BD40, with temperature controls.
- 3.1.2. Automated sampler.
- 3.1.3. Proportioning pump.
- 3.1.4. Colorimeter with 880 nm filters, 199-B021-04 phototubes, 5 cm flow cell.
- 3.1.5. Voltage regulator.
- 3.1.6. Chart recorder.

3.2. Pump tubing and assorted manifold glassware as in Figures 7.

3.3. Culture tubes, 18 x 50 mm and racks.

3.4. Digestion tubes, 25 x 200 mm calibrated at 25 and 50 ml.

3.5. Digestion tube racks to fit block digester.

3.6. Pipettors.

3.7. Vortex mixer.

#### 4. Reagents

4.1. Potassium dihydrogen phosphate ( $\text{KH}_2\text{PO}_4$ ), anhydrous, reagent grade powder.

4.2. Sulphuric acid ( $\text{H}_2\text{SO}_4$ ), concentrated reagent grade.

- 4.3. Ammonium molybdate tetrahydrate  $((\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O})$ , reagent grade crystals.
- 4.4. Antimony potassium tartrate semi-hydrate  $(\text{K}(\text{SbO})\text{C}_2\text{H}_4\text{O}_6 \cdot \frac{1}{2}\text{H}_2\text{O})$ , reagent grade crystals.
- 4.5. Sodium chloride ( $\text{NaCl}$ ), reagent grade crystals.
- 4.6. Ascorbic acid ( $\text{C}_6\text{H}_8\text{O}_6$ ), reagent grade crystals.
- 4.7. Hydrochloric acid ( $\text{HCl}$ ), concentrated reagent grade.
- 4.8. Tetra-sodium pyrophosphate ( $\text{Na}_4\text{P}_2\text{O}_7$ ), anhydrous reagent grade powder.

**4.9. Digestion Acid**

Dissolve 10.0 g mercuric oxide in 100 ml 20% sulphuric acid. Carefully add 1 liter concentrated sulphuric acid to 3 liters of distilled, deionized water. When well mixed, add 670 g potassium sulphate and dissolve. Add mercuric oxide solution and 900 ml distilled, deionized water to the sulphuric acid - potassium sulphate solution. Mix well and store above  $20^\circ\text{C}$ .

**NOTE:** Wear gloves and safety glasses when preparing this solution.

**4.10. Sulphuric Acid (20% v/v)**

Add 1600 ml concentrated sulphuric acid to 6400 ml distilled, deionized water. Add the acid in 500 ml increments while stirring. Cool in sink of cold running water.

**NOTE:** Wear gloves and safety glasses when preparing this solution.

**4.11. Hydrochloric Acid (10% v/v)**

Add 400 ml concentrated hydrochloric acid to 3.6 liters distilled, deionized water.

**NOTE:** Wear gloves and safety glasses when preparing this solution.

**4.12. Anti-bumping Granules**

Wash well with 10% hydrochloric acid. Rinse several times with distilled, deionized water and dry overnight.

**4.13. Wash Water**

Dilute 300 ml of 20% sulphuric acid to 4 liters with distilled, deionized water. Add 53.6 g potassium sulphate (without rinsing) and dissolve.

**4.14. Ammonium Molybdate Solution**

Dissolve 48 g ammonium molybdate tetrahydrate in 1 liter distilled, deionized water. Add 1000 ml 20% sulphuric acid while stirring. Cool and add 1.00 g antimony potassium tartrate. Dissolve and dilute to 4.0 liters.

**4.15. Ascorbic Acid Solution**

Dissolve 20 g ascorbic acid in distilled, deionized water and dilute to 1 liter. Although this solution is sufficiently stable for 3 consecutive days, daily preparation is recommended.

**4.16. Sodium Chloride (1% w/v)**

Dissolve 10 g sodium chloride in 1 liter distilled, deionized water.

**4.17. Quality Control Stock Solution (100 mg/l P)**

Dissolve 0.8788 g of potassium dihydrogen phosphate in distilled, deionized water and dilute to 2 liters in a volumetric flask. Store in a refrigerator.

**4.18. Quality Control Intermediate Solution (10 mg/l P)**

In a volumetric flask, dilute 100 ml of the 100 mg/l P stock solution to 1 liter with distilled, deionized water. Store this solution in specially prepared ampules.

**4.19. Quality Control Working Solutions**

QC-A: In a volumetric flask, dilute 30 ml of the 10 mg/l P intermediate solution to 2 liters with fresh wash water. This gives a phosphorus concentration of 150 mg/l P.

QC-B: In a volumetric flask, dilute 10 ml of the 10 mg/l P stock solution to 2 liters with fresh wash water. This gives a phosphorus concentration of 50 mg/l P.

Long Term Blank: Retain 2 liters of the fresh wash water solution which was used to prepare QC-A and QC-B.

**4.20. Calibration Stock Solution (100 mg/l P)**

Dissolve 0.8788 g of potassium dihydrogen phosphate in distilled, deionized water and dilute to 2 liters. Store this solution in a refrigerator.

**4.21. Calibration Intermediate Solution (4 mg/l P)**

In a volumetric flask, dilute 40 ml of the 100 mg/l P stock solution to 1 liter with distilled, deionized water.

**4.22. Calibration Working Solutions**

High: In a volumetric flask, dilute 40 ml of the 4 mg/l P intermediate solution to 1 liter with fresh wash water. This gives a phosphorus concentration of 0.160 mg/l P.

Low: In a volumetric flask, dilute 5 ml of the 4 mg/l P intermediate solution to 1 liter with fresh wash water. This gives a phosphorus concentration of 0.020 mg/l P. Prepare weekly.

**4.23. Recovery Super Stock Solution (280 mg/l P)**

Dissolve 1.2018 g of tetra sodium pyrophosphate in distilled, deionized water and dilute to 1 liter. Store this solution in a refrigerator.

**4.24. Recovery Stock Solution (28 mg/l P)**

In a volumetric flask, dilute 100 ml of the super stock solution to 1 liter with distilled, deionized water.

**4.25. Recovery Intermediate Solution (0.140 mg/l P)**

In a volumetric flask, dilute 10 ml of the 28 mg/l P stock solution to 2 liters with distilled, deionized water. Prepare weekly.

#### 4.26. Recovery Working Solutions

- $R_1$ : Pipet 25 ml of the intermediate solution into a digestion tube. Digest and dilute to 25 ml with distilled, deionized water. This gives a phosphorus concentration of 0.140 mg/l P.
- $R_2$ : Pipet 15 ml of the intermediate solution into a digestion tube. Digest and dilute to 25 ml with distilled, deionized water. This gives a phosphorus concentration of 0.084 mg/l P.
- $R_3$ : Pipet 5 ml of the intermediate solution into a digestion tube. Digest and dilute to 25 ml with distilled, deionized water. This gives a phosphorus concentration of 0.028 gm/l P.

Digested Blank: Digest 2.5 ml of digestion acid and dilute to 25 ml with distilled, deionized water.

## 5 Procedure

### 5.1. Sample Preparation

- 5.1.1. Collect samples and group them according to the bench sheet.
- 5.1.2. Mix the sample well and pipet 25 ml (before settling of particulate matter) into a clean digestion tube which has been rinsed with distilled, deionized water. Repeat for all samples and standards, rinsing the pipet between aliquots.
- 5.1.3. Add 2.5 ml of digestion acid to each tube using a precalibrated pipettor.
- 5.1.4. Add 5 or 6 boiling chips to each tube.
- 5.1.5. Vortex each tube to ensure a homogeneous solution and then place the tubes in the digestion rack in the same order.
- 5.1.6. Digest samples in the block digester at 190 °C until the water from all samples has been expelled. This step takes about 40 minutes.
- 5.1.7. Remove the rack from the 190 °C block digester and place it directly into the high temperature block digester at 360 °C to fume for exactly 20 minutes.
- 5.1.8. On completion of the digestion stage, place the rack of tubes on an asbestos mat to cool.
- 5.1.9. Allow the samples to cool sufficiently before diluting to 25 ml with distilled, deionized water with a pre-set pipettor.
- 5.1.10. Vortex the samples to mix them completely and transfer the sample to a tube for analysis in an AutoAnalyzer system.
- 5.1.11. If any carbon particles remain, discard the sample and repeat using a higher dilution factor.
- 5.1.12. Place the sampling tubes into a test tube rack in such a position that it may easily be correlated with the sample number on the bench sheet. Write the appropriate sample number on the first and last sample tube in each row.



#### NOTES:

1. Do not let any tube boil over. If activity is excessive, lift the tube out for a few seconds, add more boiling chips and replace.
2. Keep the fumehood window down as far as possible in case of an explosion.
3. Always wear gloves and safety glasses.

#### 5.2. Phosphorus Determination

- 5.2.1. Set the AutoAnalyzer into operation using cleaning, set-up and checking procedures appropriate to the manifold illustrated in Figure 7.
- 5.2.2. When loading the samples into the AutoAnalyzer sampling modules ensure that the sample order conforms to the bench sheet.
- 5.2.3. Each run of samples will include all of the following units:  
Set of calibration standards: H, L  
Wash Water Blank: Bl  
Quality Control Standards: QC-A, QC-B  
Long term blank: LTB  
Recovery solutions: R<sub>1</sub>, R<sub>2</sub>, R<sub>3</sub>  
Digested Blank: DB  
The basic loading sequence is: 10 samples, Bl, 10 samples, L, H, Bl.
- 5.2.4. Calibrate the AutoAnalyzer system using the working calibration standards. Record the calibration setting and check to see that it has not changed greatly from the previous day.
- 5.2.5. Confirm the calibration by analyzing the Quality Control solutions. Record these values. Calculate QC-A plus QC-B and QC-A minus QC-B. Check to see that these calculated values conform to limits based on past data.

#### Summary

Solution	Digested Yes/No	Acceptable Reading mg/l P
H	No	0.160
L	No	0.020
Bl	No	0
QC-A	No	0.150
QC-B	No	0.050
LTB	No	<0.002
R <sub>1</sub>	Yes	0.140
R <sub>2</sub>	Yes	0.084
R <sub>3</sub>	Yes	0.024
DB	Yes	<0.002

- 5.2.6. Monitor calibration standards (in-run sensitivity checks) throughout the run to determine if within-run corrections are required.

- 5.2.7. Read sample peak heights and convert to concentration values.
- 5.2.8. Any sample readings which are too high for the scale must be diluted with fresh wash water. Another way to run high samples is to pipet smaller aliquots before digesting.

Factor	Volume pipetted ml
1	25
2½	10
5	5

## 6. Calculation and Reporting

If required, multiply the reading by the following dilution factor:

$$\text{dilution factor} = \frac{\text{diluted volume}}{\text{aliquot volume}}$$

Record the result in the answer space opposite each sample number on the bench sheet. Report the results to the nearest 0.001 mg/l P if feasible.

## 7. Precision and Accuracy

Within-run standard deviations based on duplicate samples are as follow:

Sample Concentrations mg/l P	Standard Deviation mg/l P
<0.040	0.0022
0.040 - 0.100	0.0038
0.100 - 0.200	0.0060
Average	0.0029

Accuracy - Recovery

Quality Control Concentrations mg/l P	Average Recovery mg/l P	Standard Deviation mg/l P
0.150	0.1512	0.0013
0.050	0.0510	0.0009
Recovery Concentrations mg/l P	Average Recovery mg/l P	Standard Deviation mg/l P
0.140	0.1414	0.0042
0.084	0.0859	0.0034

## 8. Bibliography

- 8.1. American Public Health Association, American Water Works Association and Water Pollution Control Federation (1975). Standard Methods for the Examination of Water and Wastewater. 14th edition APHA, Washington, D.C. 624-628.

- 8.2. Crowther, J. and Wright, B. (1978). Ascorbic Acid Reduction Procedure for Phosphate Analysis. Ministry of the Environment, Internal Report, Laboratory Services Branch, Rexdale, Ontario.
- 8.3. Going, J. E. and Eisenreich, S. J. (1974). Spectrophotometric studies of reduced molybdoantimonylphosphoric acid. *Analytica Chimica Acta* **70**: 95-106.
- 8.4. Jirka, A. M., Carter, M. J., May, D. and Fuller, F. D. (1976). Ultramicro semi-automated method for simultaneous determination of total phosphorus and total Kjeldahl nitrogen in wastewaters. *Environmental Science and Technology* **10**: 1038-1044.
- 8.5. Murphy, J. and Riley, J. P. (1962). A modified single solution method for the determination of phosphate in natural water. *Analytica Chimica Acta* **27**: 31-36.
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## TOTAL PHOSPHORUS

### Acid Digestion - Automated Ascorbic Acid Colorimetry Method C - Variation 1

#### SUMMARY

<b>Matrix.</b>	This method is used for total phosphorus determinations on sewage and industrial waste.
<b>Substance determined.</b>	Total phosphorus, P.
<b>Interpretation of results.</b>	Results are reported in mg/l P.
<b>Principle of method.</b>	Samples are digested in sulphuric acid-mercuric oxide-potassium sulphate media using 2 block digesters maintained at 200 <sup>o</sup> C and 360 <sup>o</sup> C. The orthophosphate content in the digestate is determined by the formation of a reduced phospho-antimonylmolybdate complex using ascorbic acid as the reducing agent. pH is controlled throughout.
<b>Time required for analysis.</b>	Samples are digested in batches of 40. Approximately 150 samples can be analyzed per day.
<b>Range of application.</b>	0.03 to 2.00 mg/l as P.
<b>Standard deviation.</b>	For sewage and industrial waste samples, the average standard deviation based on within run duplicates is 0.0330 mg/l P. More detailed information is provided in Section 7, Precision and Accuracy.
<b>Accuracy.</b>	Recoveries for two Quality Control solutions were 94% and 100.4%. Recoveries for two recovery solutions were 93% and 98%. Relative standard deviations ranged from 0.77% to 5.5%.
<b>Detection criteria.</b>	0.054 mg/l P.
<b>Interferences and shortcomings.</b>	Arsenate above 300 µg/l, ferric iron above 500 mg/l and silicate above 500 mg/l interfere.
<b>Minimum volume of sample.</b>	75 ml.
<b>Sample container and preservation.</b>	Samples should be submitted in glass or polystyrene containers.
<b>Safety considerations.</b>	Exercise caution when handling concentrated acids.

## TOTAL PHOSPHORUS

### Acid Digestion - Automated Ascorbic Acid Colorimetry Method C

#### 1. Introduction

Samples are digested in sulphuric acid-mercuric oxide-potassium sulphate media using 2 block digesters maintained at 200°C and 360°C. Using an AAI system, within-run pH control is obtained and orthophosphate is measured by the formation of a reduced phospho-antimonymolybdate complex using ascorbic acid as the reducing agent.

#### 2. Interferences and Shortcomings

Interferences include arsenate above 25 µg/l, ferric iron above 50 mg/l and silicate above 50 mg/l.

#### 3. Apparatus

3.1. Auto Analyzer AAI system with the following modules:

- 3.1.1. 2 Block digestors, BD40, with temperature controls.
- 3.1.2. Automated sampler.
- 3.1.3. Proportioning pump.
- 3.1.4. Colorimeter with 880 nm filters, 199-B021-04 phototubes, 5 cm flow cell.
- 3.1.5. Voltage regulator.
- 3.1.6. Chart recorder.

3.2. Pump tubing and assorted manifold glassware as in Figures 8.

3.3. Culture tubes, 18 x 50 mm and racks.

3.4. Digestion tubes, 25 x 200 mm calibrated at 25 and 50 ml.

3.5. Digestion tube racks to fit block digester.

3.6. Pipettors.

3.7. Vortex mixer.

#### 4. Reagents

4.1. Potassium dihydrogen phosphate ( $\text{KH}_2\text{PO}_4$ ), anhydrous, reagent grade powder.

4.2. Sulphuric acid ( $\text{H}_2\text{SO}_4$ ), concentrated reagent grade.

- 4.3. Ammonium molybdate tetrahydrate  $((\text{NH}_4)_6 \text{Mo}_7 \text{O}_{24} \cdot 4\text{H}_2\text{O})$ , reagent grade crystals.
- 4.4. Antimony potassium tartrate semi-hydrate  $(\text{K}(\text{SbO})\text{C}_2\text{H}_4\text{O}_6 \cdot \frac{1}{2}\text{H}_2\text{O})$ , reagent grade crystals.
- 4.5. Sodium chloride ( $\text{NaCl}$ ), reagent grade crystals.
- 4.6. Ascorbic acid ( $\text{C}_6\text{H}_8\text{O}_6$ ), reagent grade crystals.
- 4.7. Hydrochloric acid ( $\text{HCl}$ ), concentrated reagent grade.
- 4.8. Tetra-sodium pyrophosphate ( $\text{Na}_4\text{P}_2\text{O}_7$ ), anhydrous reagent grade powder.

**4.9. Sulphuric Acid (20% v/v)**

Add 1600 ml concentrated sulphuric acid to 6400 ml distilled, deionized water. Add the acid in 500 ml increments while stirring. Cool in sink of cold running water.

**NOTE:** Wear gloves and safety glasses when preparing this solution.

**4.10. Digestion Acid**

Dissolve 10.0 g mercuric oxide in 100 ml 20% sulphuric acid. Carefully add 1 liter concentrated sulphuric acid to 3 liters of distilled, deionized water. While the solution is still hot, add 670 g potassium sulphate and dissolve. Add mercuric oxide solution to the sulphuric acid - potassium sulphate solution and dilute to 5 liters with distilled, deionized water. Mix well and store above  $20^\circ\text{C}$ .

**NOTE:** Wear gloves and safety glasses when preparing this solution.

**4.11. Hydrochloric Acid (10% v/v)**

Add 400 ml concentrated hydrochloric acid to 3.6 liters distilled, deionized water.

**NOTE:** Wear gloves and safety glasses when preparing this solution.

**4.12. Anti-bumping Granules**

Wash well with 10% hydrochloric acid. Rinse several times with distilled, deionized water and dry overnight.

**4.13. Wash Water**

Dilute 52 ml of concentrated sulphuric acid to 3 liters with distilled, deionized water. Add 48 g potassium sulphate and dilute to 4.5 liters with distilled, deionized water..

**NOTE:** Wear gloves and safety glasses when preparing this solution.

**4.14. Ammonium Molybdate Solution**

Dissolve 48 g ammonium molybdate tetrahydrate in 1 liter distilled, deionized water. Add 480 ml of concentrated sulphuric acid while stirring. Cool and add 1.00 g antimony potassium tartrate. Dissolve and dilute to 4.0 liters.

**NOTE:** Wear gloves and safety glasses when preparing this solution.

#### 4.15. Ascorbic Acid Solution

Dissolve 20 g ascorbic acid in distilled, deionized water and dilute to 1 liter. Although this solution is sufficiently stable for 3 consecutive days, daily preparation is recommended.

#### 4.16. Sodium Chloride (1% w/v)

Dissolve 10 g sodium chloride in 1 liter distilled, deionized water.

#### 4.17. Sodium Hydroxide (6.2N)

Dissolve 248 g sodium hydroxide in 1 liter of distilled, deionized water.

**NOTE:** Wear gloves and eye protection when preparing this solution.

#### 4.18. Dilution Water

Dissolve 5 g of sodium chloride in 5 liters of distilled, deionized water.

#### 4.19. Quality Control Stock Solution (56 mg/l P)

Dissolve 0.2462 g of potassium dihydrogen phosphate in distilled, deionized water and dilute to 1 liter in a volumetric flask. Keep this solution refrigerated..

#### 4.20. Quality Control Working Solutions

QC-A: In a volumetric flask, dilute 50 ml of the 56 mg/l P stock solution to 2 liters with fresh wash water. This gives a phosphorus concentration of 1.4 mg/l P.

QC-B: In a volumetric flask, dilute 10 ml of the 56 mg/l P stock solution to 2 liters with fresh wash water. This gives a phosphorus concentration of 0.28 mg/l P.

Long Term Blank: Retain 2 liters of the fresh wash water solution used to prepare QC-A and QC-B.

#### 4.21. Recovery Stock Solution (112 mg/l P)

Dissolve 0.4803 g of tetra sodium pyrophosphate in 800 ml of deionized distilled water. Adjust the pH to 9.5 and dilute to 1 liter with deionized distilled water. Keep this solution refrigerated.

#### 4.22. Recovery Working Solutions

R<sub>1</sub>: Transfer 50 ml of the 112 mg/l P recovery stock solution to a 2 liter volumetric flask. Adjust the pH to 9.5 with 6.2N sodium hydroxide if necessary and dilute to 2 liters with distilled, deionized water. Digest 25 ml and dilute to 50 ml with distilled, deionized water. This gives a phosphorus concentration of 1.4 mg/l P.

R<sub>2</sub>: Transfer 25 ml of the 112 mg/l P recovery stock solution to a 2 liter flask and adjust the pH the 9.5 with 6.2N sodium hydroxide if necessary. Dilute to 2 liters with distilled, deionized water. Digest 25 ml and dilute to 50 ml with distilled, deionized water. This gives a phosphorus concentration of 0.7 mg/l P.

#### 4.23. Calibration Stock Solution (320 mg/l P)

Dissolve 1.406 g of potassium dihydrogen phosphate in 500 ml of distilled, deionized water. Dilute to 1 liter. Store this solution in an actinic glass bottle.

#### 4.24. Calibration Intermediate Solution (16 mg/l P)

In a volumetric flask, dilute 50 ml of the 320 mg/l P stock solution to 1 liter with distilled, deionized water.

#### 4.25. Calibration Working Solutions

High: In a volumetric flask, dilute 100 ml of the 16 mg/l P intermediate solution to 1 liter with fresh wash water. This gives a phosphorus concentration of 1.6 mg/l P.

### 5. Procedure

#### 5.1. Sample Preparation

- 5.1.1. Collect samples and group them according to the bench sheet.
- 5.1.2. Mix the sample well and pipet 25 ml (before settling of particulate matter) into a clean digestion tube which has been rinsed with distilled, deionized water. Repeat for all samples and standards, rinsing the pipet between aliquots.
- 5.1.3. Add 4.0 ml of digestion acid to each tube using a precalibrated pipettor.
- 5.1.4. Add 5 or 6 boiling chips to each tube.
- 5.1.5. Vortex each tube to ensure a homogeneous solution and then place the tubes in the digestion rack in the same order.
- 5.1.6. Digest samples in the block digester at 200°C until the water from all samples has been expelled. This step takes about 40 minutes.
- 5.1.7. Remove the rack from the 200°C block digester and place it directly into the high temperature block digester at 360°C to fume for exactly 20 minutes.
- 5.1.8. On completion of the digestion stage, place the rack of tubes on an asbestos mat to cool.
- 5.1.9. Allow the samples to cool sufficiently before diluting to 50 ml with distilled, deionized water with a pre-set pipettor.
- 5.1.10. Vortex the samples to mix them completely and transfer the sample to a tube for analysis in an AutoAnalyzer system.
- 5.1.11. If any carbon particles remain, discard the sample and repeat using a higher dilution factor.
- 5.1.12. Place the sampling tubes into a test tube rack in such a position that it may easily be correlated with the sample number on the bench sheet. Write the appropriate sample number on the first and last sample tube in each row.

#### NOTES:

1. Do not let any tube boil over. Lift the tube out for a few seconds, add new boiling chips and replace.



2. Keep the fumehood window down as far as possible in case of an explosion.
3. Always wear gloves and safety glasses.

## 5.2. Phosphorus Determination

- 5.2.1. Set the AutoAnalyzer into operation using cleaning, set-up and checking procedures appropriate to the manifold illustrated in Figure 8.
- 5.2.2. When loading the samples into the AutoAnalyzer sampling modules ensure that the sample order conforms to the bench sheet.
- 5.2.3. Each run of samples will include all of the following units:  
 Set of calibration standards: H  
 Wash Water Blank: Bl  
 Quality Control Standards: QC-A, QC-B  
 Long term blank: LTB  
 Recovery solutions:  $R_1$ ,  $R_2$   
 Digested Blank: DB  
 The basic loading sequence is: 10 samples, Bl, 10 samples, L, H, Bl.
- 5.2.4. Calibrate the AutoAnalyzer system using the working calibration standards. Record the calibration setting and check to see that it has not changed greatly from the previous day.
- 5.2.5. Confirm the calibration by analyzing the Quality Control solutions. Record these values. Calculate QC-A plus QC-B and QC-A minus QC-B. Check to see that these calculated values conform to limits based on past data.

### Summary

Solution	Digested Yes/No	Acceptable Reading mg/l P
H	No	1.60
Bl	No	0
QC-A	No	1.40
QC-B	No	0.28
LTB	No	<0.002
$R_1$	Yes	1.40
$R_2$	Yes	0.70
DB	Yes	<0.002

- 5.2.6. Monitor calibration standard (in-run sensitivity checks) throughout the run to determine if within-run corrections are required.
- 5.2.7. Read sample peak heights and convert to concentration values.
- 5.2.8. Any sample readings which are too high for the scale must be diluted with fresh washwater. Another way to run high samples is to pipet smaller aliquots before digesting.

Factor	Volume pipetted ml
1	25
2½	10
5	5

## 6. Calculation and Reporting

If required, multiply the reading by the following dilution factor:

$$\text{dilution factor} = \frac{\text{diluted volume}}{\text{aliquot volume}}$$

Record the result in the answer space opposite each sample number on the bench sheet. Report the results to the nearest 0.01 mg/l P if feasible.

## 7. Precision and Accuracy

Precision:

Within-run standard deviations based on duplicate samples are as follow:

Sample Concentrations mg/l P	Standard Deviation mg/l P
<0.04	0.0201
0.04 - 1.00	0.0304
1.00 - 2.00	0.0573
Average	0.0330

Accuracy - Recovery

Quality Control Concentrations mg/l P	Average Recovery mg/l P	Standard Deviation mg/l P
1.40	1.406	0.0109
0.28	0.262	0.0161
Recovery Concentrations mg/l P	Average Recovery mg/l P	Standard Deviation mg/l P
1.40	1.375	0.0337
0.70	0.651	0.0389

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- 8.1. American Public Health Association, American Water Works Association and Water Pollution Control Federation (1975). Standard Methods for the Examination of Water and Wastewater. 14th edition APHA, Washington, D.C. 624-628.

- 8.2. Crowther, J. and Wright, B. (1978). Ascorbic Acid Reduction Procedure for Phosphate Analysis. Ministry of the Environment, Internal Report, Laboratory Services Branch, Rexdale, Ontario.
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## RADIOACTIVE PHOSPHORUS

### Liquid Scintillation Counting Method D

#### SUMMARY

<b>Matrix.</b>	This method is used for $^{32}\text{P}$ determinations on surface waters.
<b>Substance determined.</b>	Radioactive $^{32}\text{P}$ (as measured on filter supports).
<b>Interpretation of results.</b>	Instrumental readings are given in counts/minute (cpm) and are used in conjunction with concurrently measured standards to determine the concentration of immediately available phosphate-like material in water. This method is used at present in tracer studies of phosphorus uptake and has wide potential application in medical and biological studies.
<b>Principle of method.</b>	Two photomultiplier tubes in coincidence are used to count the scintillations caused by $\beta$ -particles emitted by $^{32}\text{P}$ contained in solid material on a filter immersed in a solvent-fluor medium.
<b>Time required for analysis.</b>	Approximately 2 minutes counting time per sample.
<b>Range of application.</b>	50 cpm and greater. Values less than 50 cpm are meaningless due to the high standard deviations encountered.
<b>Standard deviation.</b>	The precision of each sample is reported in terms of the "2 sigma percent error", determined from counting statistics. In practice the value of this parameter seldom exceeds 10%.
<b>Accuracy.</b>	Not yet determined.
<b>Detection criteria.</b>	Samples producing counting rates less than 50 cpm have 2 sigma percent errors greater than 20% and should be considered to be below the minimum detection limit.
<b>Interferences and shortcomings.</b>	None encountered.
<b>Minimum volume of sample.</b>	Not applicable.
<b>Preservation and sample container.</b>	The sample is contained in a liquid scintillation medium in a polyethylene vial with no special precautions taken for preservation.
<b>Safety considerations.</b>	Normal procedures for dealing with weakly radioactive substances.

## RADIOACTIVE PHOSPHORUS

### Liquid Scintillation Counting Method D

#### 1. Introduction

The rate of uptake of biologically available phosphate-like material by aquatic plants can be studied by liquid scintillation counting of the radioactive  $^{32}\text{P}$  activity present in the solid fraction of periodic samples taken from a  $^{32}\text{PO}_4$ -spiked dispersion of algae in water.

The scintillation process comprises a number of steps, initiated by the emission of a  $\beta$ -particle from the isotope. This particle loses energy to the molecules of a suitable aromatic solvent which in turn transfer energy (perhaps via other solvent molecules) to a scintillator solute (fluor). The light emitted when each solute molecule relaxes is detected by a pair of photomultiplier tubes and the number of pulses (counts per minute) gives an indirect measure of the radioactivity present.

Instrumental readings in counts/minute (cpm) are used in conjunction with measurements of standards to determine the percentage of  $^{32}\text{P}$  remaining in solution.

#### 2. Interferences and Shortcomings

Successful counting of samples labelled with  $\beta$ -emitting isotopes requires that the labelled sample be in close contact (on a molecular scale) with the "cocktail" (the solution containing aromatic solvent molecules and fluors) and that the effects of factors such as scintillation quenching and sample-cocktail heterogeneity be minimized. Methods for achieving these conditions are described by Long (8.1).

The major problem associated with liquid scintillation counting on filter supports as documented by Long (8.2) is the effect of the orientation of the filter paper on the number of pulses observed by the detection system. However, it appeared that for the highly energetic  $\beta$ -particles characteristic of  $^{32}\text{P}$  decay, the filter medium was essentially transparent.

#### 3. Apparatus

- 3.1. Liquid scintillation counter.
- 3.2. Vials, polyethylene, 20 ml capacity.
- 3.3. Filters, Millipore,  $0.45\ \mu\text{m}$  pore size, cellulose acetate.
- 3.4. Funnel, Millipore, stainless steel, 47 mm diameter.

#### 4. Reagents

- 4.1. Scintillation solvent system, e.g. Beckman Readi Solv, suitable for aqueous samples.
- 4.2.  $^{32}\text{P}$  tracer as  $2\ \mu\text{Ci } ^{32}\text{PO}_4$  /ml distilled, deionized water.

## 5. Procedure

- 5.1. At frequent intervals up to 15 minutes, pipette 10 ml samples of algal medium spiked with  $^{32}\text{PO}_4$  and filter through a double layer of filters.
- 5.2. Place each filter in a polyethylene vial and add a standard volume of "cocktail" (13.5 ml will fill the vial but smaller volumes have been found to give satisfactory results). Cap securely.
- 5.3. Prepare quadruplicate standards containing 1 ml  $^{32}\text{PO}_4$  solution in the standard volume of cocktail.
- 5.4. Place a sample batch (consisting of vials containing first and second filters arranged alternately followed by the 4 standards) in the sample-changing belt of the liquid scintillation counter.
- 5.5. Set the lower and upper windows of the pulse height analysis system at 5 and 100% of the total discriminator width respectively and count each sample for 2 minutes or until the "2 sigma percent error" (vide infra) drops to 0.2%, whichever is shorter.

## 6. Calculation and Reporting

- 6.1. Results are printed out as counts per minute (cpm) with an associated standard deviation.
- 6.2. The amount of phosphate taken up during a given time interval can be determined as follows:
  - 6.2.1. Subtract count measured for the second filter paper from that obtained for the first. This accounts for the radioactivity in the sample arising as a result of  $\text{PO}_4^{3-}$  adsorption on the filter.
  - 6.2.2. No quenching correction is required for the highly energetic  $\beta$  particles resulting from  $^{32}\text{P}$  decay.
  - 6.2.3. Determine %  $^{32}\text{P}$  taken up according to the following:

$$\% ^{32}\text{P} = \frac{100 \times \text{net cpm}}{\text{standard cpm}}$$

## 7. Precision and Accuracy

A "2 sigma percent error", defined as  $200/\sqrt{N}$ , where N is the total number of counts observed, is calculated and printed for each sample. The significance of this error is that 95.5% of subsequent remeasurements would be expected to be within the quoted margin. It must of course be borne in mind that this represents the possible error in counting only and does not include other errors (e.g. pipetting, weighing) accrued during sample preparation.

The accuracy of the method has not yet been determined.

## 8. Bibliography

- 8.1 Long, E. C. (1976). Selective Aspects of Sample Handling in Liquid Scintillation Counting. Report 1042-NUC-76-55T, Beckman Instruments, Inc. Fullerton CA.
- 8.2 Long, E. C. (1976). Liquid Scintillation Counting on Filter Supports. Report 916-NUC-76-8T Beckman, Instruments, Inc. Fullerton CA.

**TOTAL PHOSPHORUS**  
**X-ray Fluorescence Method E**

**SUMMARY**

<b>Matrix.</b>	This method is used routinely on vegetation samples.
<b>Substance determined.</b>	Phosphorus, P.
<b>Interpretation of results.</b>	Results are reported as % or $\mu\text{g/g}$ of dry weight of vegetation.
<b>Principle of method.</b>	The X-ray fluorescence is measured at $133.27^0$ using the graphite crystal. The intensity is related to the concentration of the element in the sample. The concentrations are corrected for matrix effects.
<b>Time required for analysis.</b>	100 samples can be weighed and pelletized in one day. 140 samples can be analyzed in one day for 6 elements.
<b>Range of application.</b>	.02% - .6%.
<b>Standard deviation.</b>	0.02% overall.
<b>Accuracy.</b>	0.01% for the 0.02-0.2% phosphorus range; 0.02% for the 0.2-0.4% range and 0.03% for the 0.4-0.6% range.
<b>Detection criteria.</b>	The detection limit is 0.02%.
<b>Interferences and shortcomings.</b>	No interference; matrix correction required.
<b>Minimum volume of sample.</b>	2 g powdered vegetation.
<b>Preservation and sample container.</b>	Store pellets in a dessicator.
<b>Safety considerations.</b>	Operator must wear radiation monitoring badge.

FOR A DETAILED DESCRIPTION OF METHOD SEE "THE DETERMINATION OF TRACE METALS".



## TOTAL PHOSPHORUS

### Digestion-Automated Molybdenum Blue Colorimetry Method F

#### SUMMARY

<b>Matrix.</b>	This method is used on soil, sediment, vegetation and dried sludge samples.
<b>Substance determined.</b>	Total phosphorus, P.
<b>Interpretation of results.</b>	Results are reported as mg/g P.
<b>Principle of method.</b>	Organic phosphorus in the samples is converted to inorganic phosphate by digestion in fuming sulphuric acid and potassium persulphate. The latter serves to raise the boiling point of the digestion mixture and to provide a highly oxidizing medium for the decomposition of organic matter. The acidic digests are neutralized to the methyl-red endpoint with sodium hydroxide, treated to remove heavy metal interference if necessary, and analyzed by addition of ammonium molybdate and stannous chloride to form the reduced heteropoly phosphomolybdate blue complex. Color development is monitored photometrically with an automated system at 660 nm.
<b>Time required for analysis.</b>	Approximately 40 samples can be prepared for analysis in one day. Phosphorus determination is performed by AutoAnalyzer at a rate of 20 samples per hour.
<b>Range of application.</b>	0 - 1.6 mg/l in solution. Samples in excess are measure by dilution.
<b>Standard deviation.</b>	Based on 44 replicate samples, standard deviations are 0.02 at the 0.4 mg/l level and 0.06 at the 1.6 mg/l phosphorus level.
<b>Accuracy.</b>	Recoveries of four recovery standards ranged from 101.9% to 102.8%. Relative standard deviations of these solutions ranged from 1.2% to 3.5%.
<b>Detection criteria.</b>	The detection limit is 0.03 mg/l.

**Interferences  
and shortcomings.**

Iron and calcium complex with phosphate under the conditions used for color development. Furthermore, Fe(III) and other oxidized heavy metals compete with the phosphomolybdate complex for the reducing agent, stannous chloride. Metal concentrations are usually too low in vegetation to cause problems, however, soils, sediments and sludges must be pretreated to precipitate hydroxides to minimize these interferences.

**Minimum volume  
of sample.**

0.02 g vegetation (dry weight) and 0.1 - 0.5 g soil or sediment (dry weight).

**Preservation and  
sample container.**

Soils, sediments and sludges may be collected in glass jars, air dried and ground to less than 2 mm. Vegetation samples are dried and ground.

**Safety  
precautions.**

Safety glasses should be worn during the digestion procedure.

## TOTAL PHOSPHORUS

### Acid Digestion -Automated Molybdenum Blue Colorimetry Method F

#### 1. Introduction

Organic phosphorus is converted to inorganic phosphate by digestion in a fuming sulphuric acid and potassium persulphate mixture. The persulphate serves to raise the boiling point and to provide an oxidizing medium for the decomposition of organic matter. The acidic digests are neutralized to the methyl-red end point with sodium hydroxide and sulphuric acid. Acidic ammonium molybdate is added to produce a phosphomolybdate complex which is reduced to the heteropoly blue species with stannous chloride. The color development is followed photometrically at 660 nm in an automated system.

Soil and sediment digests are treated for heavy metal interference by addition of disodium EDTA to an aliquot of the original digest followed by addition of excess sodium hydroxide in order to precipitate the metal hydroxides, which are filtered out. The filtrate is adjusted to the methyl-red end point with sulphuric acid and analyzed as described above.

#### 2. Interferences and Shortcomings

Heavy metals, especially iron, and calcium interfere with color development by the formation of complexes with the phosphate in the sample. In addition (Fe (III) competes with phosphomolybdate for the reducing agent, stannous chloride. These interferences are overcome by precipitation of the interferents as hydroxides followed by filtration.

#### 3. Apparatus

##### 3.1. AutoAnalyzer II system with the following modules:

- 3.1.1. Sampler
- 3.1.2. Proportioning Pump
- 3.1.3. Colorimeter equipped with 660 nm filters and a 5 cm flow cell
- 3.1.4. Voltage regulator
- 3.1.5. Chart recorder.

##### 3.2. Pump tubing and assorted manifold glassware as in Figure 9.

- 3.3. Culture tubes, 19 x 105 mm.
- 3.4. Test Tube racks, 40 tube capacity.
- 3.5. Balance, Sartorius Model 3705, top loading or equivalent.
- 3.6. Hot plate (heavy duty), Lindberg or equivalent, with temperature control.
- 3.7. Erlenmeyer, 125 ml, Pyrex, calibrated  $\pm 5\%$ .
- 3.8. Filter paper, glass fibre, 4.25 cm Whatman.
- 3.9. Filtering assembly, vacuum (Buchner).
- 3.10. Brass scoop, to contain approximately 1 g of potassium persulphate.
- 3.11. Pipettor, Oxford or equivalent dispenser.
- 3.12. Burette, 50 ml.

#### 4. Reagents

- 4.1. Potassium dihydrogen orthophosphate ( $\text{KH}_2\text{PO}_4$ ), reagent grade powder.
- 4.2. Ammonium chloride ( $\text{NH}_4\text{Cl}$ ), reagent grade powder.
- 4.3. Sodium hydroxide ( $\text{NaOH}$ ), reagent grade pellets.
- 4.4. Potassium persulphate ( $\text{K}_2\text{S}_2\text{O}_8$ ), reagent grade crystals (low phosphorus).
- 4.5. EDTA, disodium salt, dihydrate ( $\text{C}_{10}\text{H}_{14}\text{N}_2\text{Na}_2\text{O}_8 \cdot 2\text{H}_2\text{O}$ ) reagent grade.
- 4.6. Sulphuric acid ( $\text{H}_2\text{SO}_4$ ) concentrated reagent grade.
- 4.7. Methyl red indicator, water soluble.
- 4.8. Ammonium molybdate tetrahydrate ( $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}$ ), reagent grade crystals.
- 4.9. Stannous chloride dihydrate ( $\text{SnCl}_2 \cdot 2\text{H}_2\text{O}$ ), reagent grade powder.
- 4.10. Hydrochloric acid ( $\text{HCl}$ ), concentrated reagent grade.
- 4.11. **Sodium Hydroxide Solution 6.25N**

Dissolve 250 g sodium hydroxide in distilled water and dilute to 1 liter.

**NOTE:** This reagent is strongly caustic. Wear eye protection during its preparation.

**4.12. EDTA Solution (5%)**

Dissolve 50 g EDTA disodium salt in distilled water and dilute to 1 liter.

**4.13. Methyl Red Indicator**

Dissolve 0.1 g water soluble methyl red in distilled water and dilute to 1 liter.

**4.14. Sulphuric Acid (10%)**

Dilute 100 ml concentrated sulphuric acid to 1 liter with distilled water. Add acid to water slowly while stirring.

**4.15. Sulphuric acid (20%)**

Dilute 200 ml concentrated sulphuric acid to 1 liter with distilled water.

**NOTE:** Wear eye protection and plastic gloves when preparing these two reagents.

**4.16. Stock Stannous Chloride Solution**

Dissolve 5 g stannous chloride dihydrate in 20 ml concentrated hydrochloric acid. Dilute to 200 ml with freshly boiled, warm deionized water. Store in a clean container with a bottom spigot. Cover the surface of the stannous chloride solution with 10 ml fresh mineral oil. Avoid exposure of this solution to the atmosphere.

**NOTE:** Wear eye protection and plastic gloves when adding the hydrochloric acid solution.

**4.17. Working Stannous Chloride Reagent**

Dilute 2 ml stock solution to 100 ml with distilled water. This reagent is sufficient for about 12 hours of continuous operation.

**4.18. Molybdate Stock Solution**

Dissolve 20 g ammonium molybdate tetrahydrate in distilled water and dilute to 1 liter.

**4.19. Working Molybdate Reagent**

Add 150 ml molybdate stock solution and 250 ml 20% sulphuric acid to 400 ml distilled water. After cooling, dilute to 1 liter with distilled water.

**NOTE:** Wear eye protection and plastic gloves when adding the sulphuric acid solution.

**4.20. Concentrated Phosphorus Stock Solution (100 mg/l P)**

Dissolve 0.4394 g anhydrous potassium dihydrogen orthophosphate in distilled water acidified with 0.25 ml concentrated sulphuric acid. Dilute to 1 liter with distilled water.

**NOTE:** Wear eye protection and plastic gloves when adding the sulphuric acid solution.

#### 4.21. Phosphorus Standard Solution (8 mg/l P)-

Dilute 80 ml of stock solution to 1 liter with distilled water.

#### 4.22. Phosphorus Calibration Standards

Dilute 5, 10, 15 and 20 ml of the phosphorus standard solution to 100 ml with distilled water. This gives a set of working standards with phosphorus concentration of 0.4, 0.8, 1.2 and 1.6 mg/l

#### 4.22. Quality Control Solutions

Prepare 2 quality control solutions at approximately 20 and 80% of range by judicious blending of at least one month's supply of sample digests.

### 5. Procedure

#### 5.1. Vegetation Digestion Procedure

- 5.1.1. Weigh approximately 0.02 g sample into a 125 ml Erlenmeyer flask. (Sample should have been previously ground in a Wiley mill, passed through a 40 mesh screen and dried for 2 hours at 105°C.)
- 5.1.2. Pipette 5, 10, 15 and 20 ml of standard phosphorus solution into similar flasks. (This will give a series of standards with 0.4, 0.8, 1.2 and 1.6 mg/l phosphorus.) These are the recovery standards  $R_1$ ,  $R_2$ ,  $R_3$  and  $R_4$ . A digestion blank should also be carried through the procedure.
- 5.1.3. To each flask, add 2 ml concentrated sulphuric acid and wash down the sides of the flask with a small amount of distilled water.
- 5.1.4. Set hot plate at highest setting possible which will not cause spattering. Digest samples and standards on the hot plate for about 45 minutes or until thick dense white fumes are generated.
- 5.1.5. Remove flasks from hot plate and cool to room temperature.
- 5.1.6. Using a scoop add 2 g potassium persulphate. Wash down persulphate with 5 ml distilled water.
- 5.1.7. Reheat flasks for 30 minutes on hot plate. Thick white fumes will be produced.
- 5.1.8. Remove flasks from the hot plate and cool to room temperature.
- 5.1.9. If sample is not completely digested, add another scoop of persulphate and redigest for another 30 minutes. Add 5 - 10 ml distilled water to each flask to dissolve the sulphate salts.

- 5.1.10. Add 4 drops methyl red indicator to each flask and titrate carefully but rapidly with 6.25N sodium hydroxide until pinkish-red color disappears. Add 8 ml of 5% EDTA solution.
- 5.1.11. Immediately back titrate the samples with 10% sulphuric acid to a pinkish red color, adding 5 drops in excess.
- 5.1.12. Dilute to 100 ml volume with distilled water.
- 5.1.13. Pour an aliquot into a test tube for colorimetric analysis by auto-analyzer as described in 5.3.

## 5.2. Soils and Sediments

- 5.2.1. Weigh out 0.08 to 0.4 g of pre-dried, pulverized sample into a 125 ml Erlenmeyer flask. (Sample weight chosen is determined by the amount of organic matter and type of sample. For example, a larger weight may be required for a sandy sample.
- 5.2.2. Pipette aliquots of standard phosphorus solutions into 125 ml Erlenmeyer flasks as in 5.1.2. and carry through digestion procedure. These are the recovery standards  $R_1$ ,  $R_2$ ,  $R_3$  and  $R_4$ .
- 5.2.3. Add 7 ml concentrated sulphuric acid using an Oxford pipettor.
- 5.2.4. Digest samples on a hot plate at high temperature for approximately 2 hours until dense white fumes are generated.
- 5.2.5. Remove flasks from hot plate after 2 hours and cool to room temperature.
- 5.2.6. Using a scoop, add approximately 2 g potassium persulphate.
- 5.2.7. Wash down persulphate with 5 ml distilled water.
- 5.2.8. Reheat flasks for 30 minutes on the hot plate. Thick white fumes will result. Digested samples should be greyish brown in color. If not, add another scoop of persulphate and digest for an additional 30 minutes.
- 5.2.9. Remove the flasks from the hot plate. Cool to room temperature and add 5 - 10 ml distilled water to dissolve the sulphate salts.
- 5.2.10. Dilute contents of flask to 100 ml and mix thoroughly by swirling.
- 5.2.11. Pipette a 25 ml aliquot of the digestate into another flask and add 4 drops methyl red indicator.
- 5.2.12. Titrate carefully but rapidly with 6.25N sodium hydroxide until pinkish red color disappears. Add 5 drops of sodium hydroxide in excess. Add 8 ml of 5% EDTA solution.

- 5.2.13. Using vacuum filtration, filter the treated digestate aliquot through a glass fibre filter. Wash the flask and filter with a few drops of 10% sodium hydroxide.
- 5.2.14. Back titrate the filtrate to the methyl-red endpoint with 10% sulphuric acid, adding 5 drops in excess.
- 5.2.15. Dilute to 100 ml volume with distilled water.
- 5.2.16. Pour an aliquot into a test tube for colorimetric analysis by auto-analyzer as described in 5.3.

### 5.3. Phosphorus Determination

- 5.3.1. Set the AutoAnalyzer into operation using the clean-up and checking procedures appropriate to the manifold illustrated in Figure 9.
- 5.3.2. When loading the samples into the AutoAnalyzer sampling module, ensure that the sample order conforms to the bench sheet.
- 5.3.3. Each sample run will include all of the following units:  
 Set of calibration standards: High, Medium, Low (H, M, L)  
 Wash Water Blank: Bl  
 Quality Control Standards: QC-A, QC-B.  
 Digested Blank: DB.  
 Recovery Standards:  $R_1$ ,  $R_2$ ,  $R_3$ ,  $R_4$ .  
 The basic loading sequence is:  
 n (10 samples, L, H, Bl).
- 5.3.4. Calibrate the AutoAnalyzer system using the calibration standards. Record the standard calibration setting and check to see that it has not changed greatly from the previous day. Construct a calibration curve.
- 5.3.5. Confirm the calibration by analyzing the Quality Control solutions. Record these values. Calculate the sums and differences and ensure that these calculated values conform to limits based on past data.
- 5.3.6. Monitor calibration standards (in run sensitivity checks) throughout the run to determine if within-run corrections are required.
- 5.3.7. Read sample peak heights and convert to concentration values.

## 6. Calculation and Reporting

Results are calculated according to the following formula:

$$C = (R(\text{mg/l}) \times 0.1 \times \text{DF})/W$$



Where:

R is the chart response in mg/l

0.1 represents the initial sample dilution in l

DF is the dilution factor (dilution volume/sample aliquot)

W is the weight of sample digested in grams

The results are reported to two significant figures as mg/g.

## 7. Precision and Accuracy

Based on 44 replicates, the standard deviation at 0.4 mg/l phosphorus is 0.02 and at 1.6 mg/l phosphorus it is 0.06.

Between run precision is controlled by 2 long term standards at 20% and 80% of range and are prepared by judicious blending of at least one month's supply of sample digests. Calibration is controlled by these standards such that QC-A and QC-B do not vary by more than 2 standard deviations as determined above, from their respective long term mean values.

### Accuracy - Recovery

Digested Standard Concentration mg/l P	Mean Recovery mg/l P	Standard Deviation mg/l
0.40	0.411	0.014
0.80	0.822	0.014
1.20	1.23	0.016
1.60	1.63	0.020

## 8. Bibliography

8.1. American Public Health Association, American Water Works Association and Water Pollution Control Federation (1971). Standard Methods for the Examination of Water and Wastewater. 13th ed. ALPHA, Washington, D.C. 518-534.

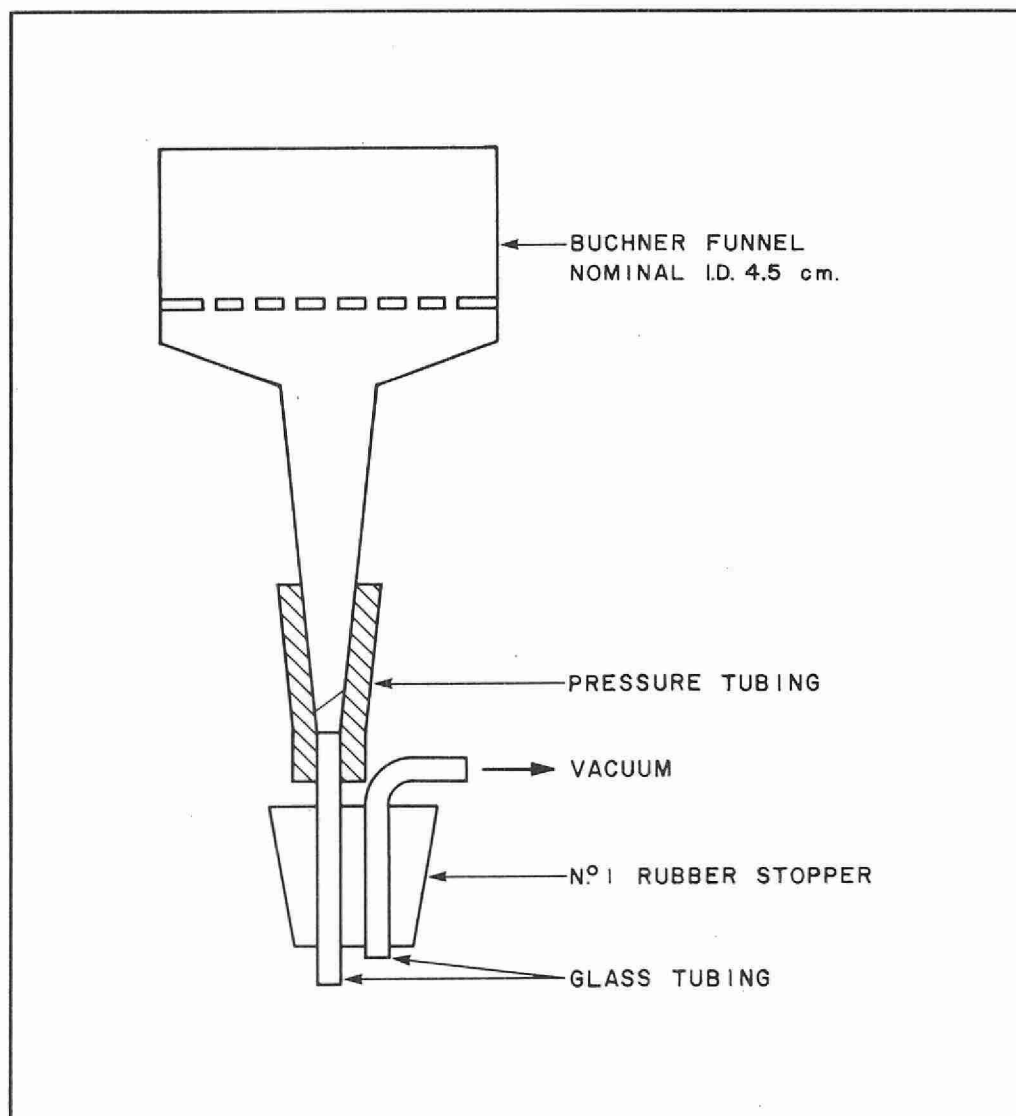


FIGURE 1 — FILTRATION APPARATUS

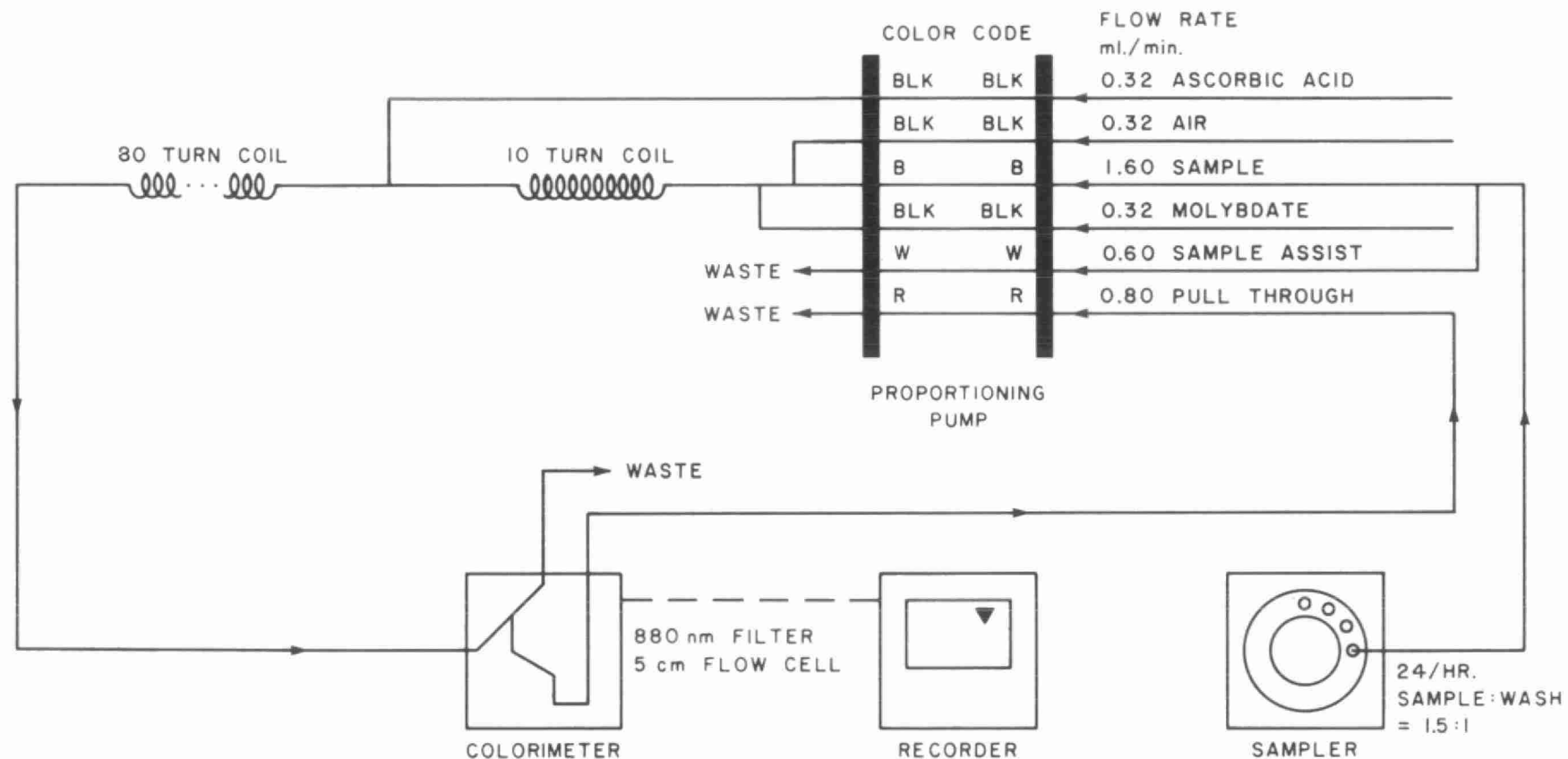
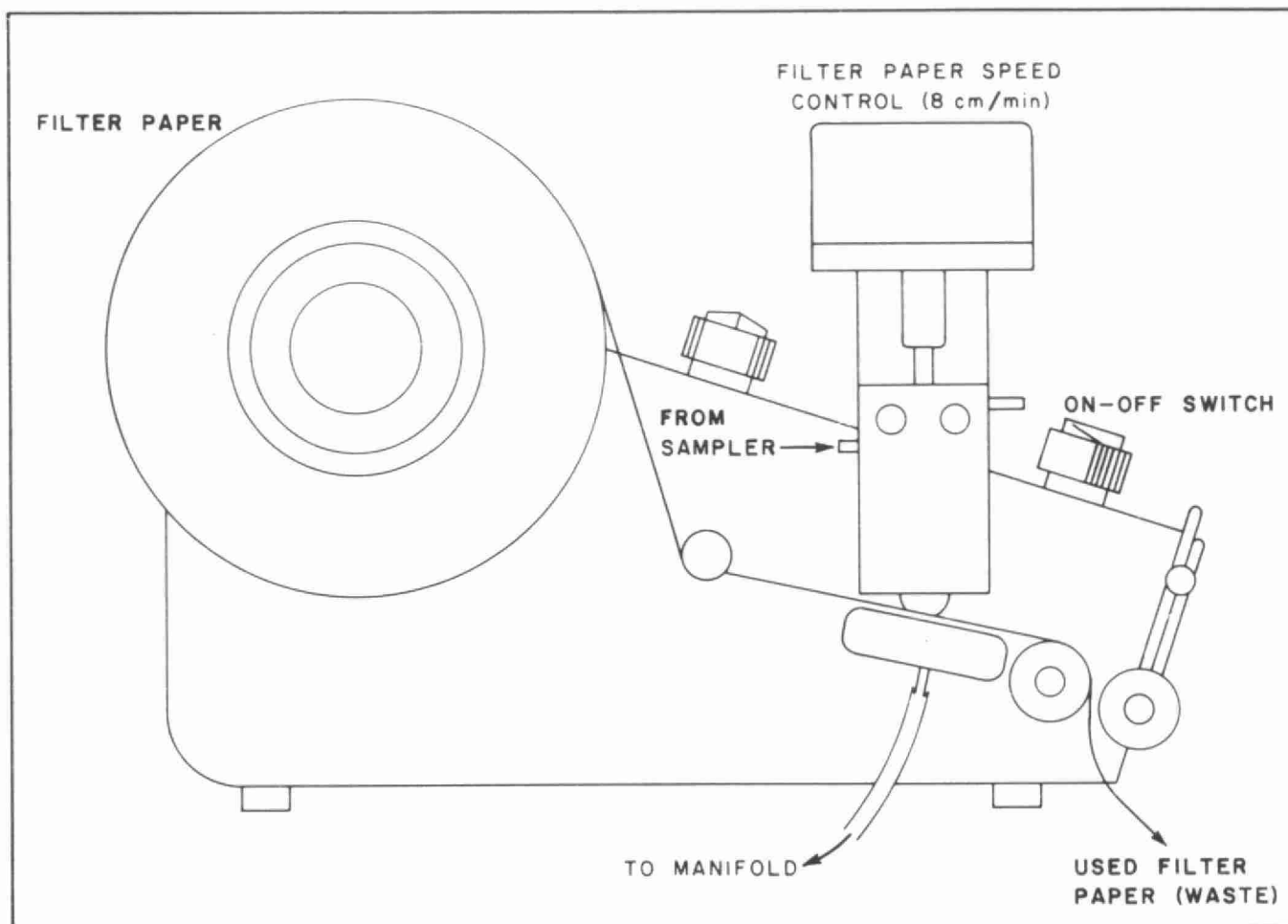


FIGURE 2 — AUTOANALYZER AAI SYSTEM FOR FILTERED REACTIVE PHOSPHORUS DETERMINATIONS IN SURFACE WATERS



**FIGURE 3 — CONTINUOUS FILTER MODULE.**

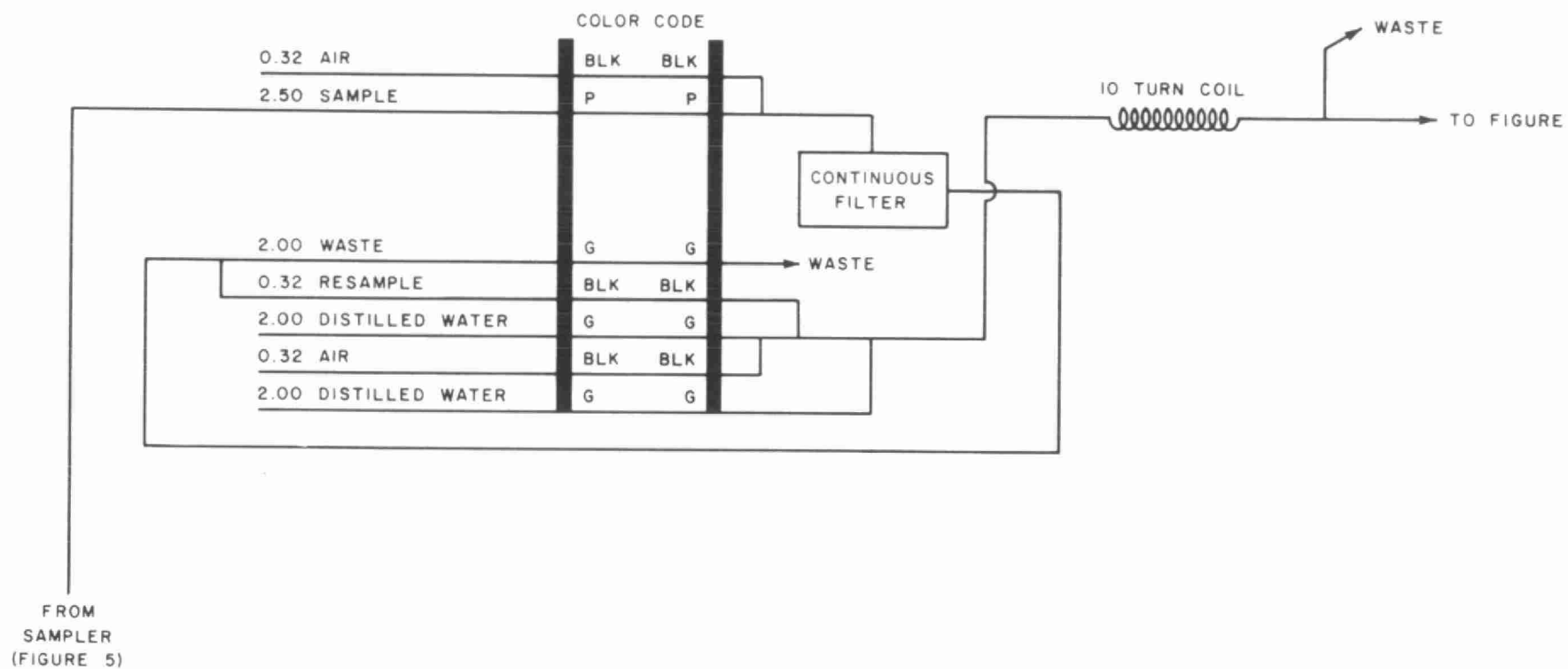


FIGURE 4 — SCHEMATIC DIAGRAM OF CONTINUOUS FILTER UNIT INCLUDING DILUTION LOOP.

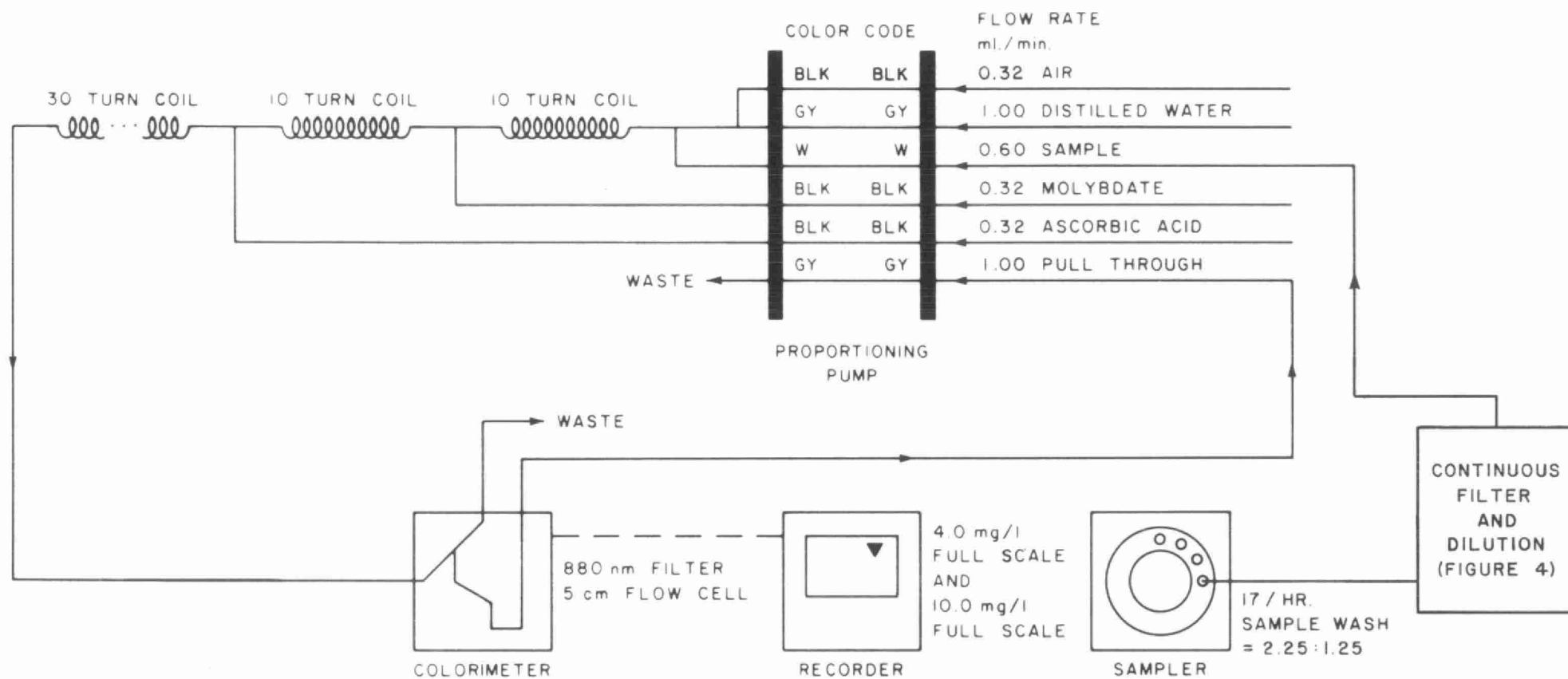


FIGURE 5 — AUTOANALYZER AAII SYSTEM FOR FILTERED REACTIVE PHOSPHORUS DETERMINATION IN SEWAGE AND INDUSTRIAL WASTE

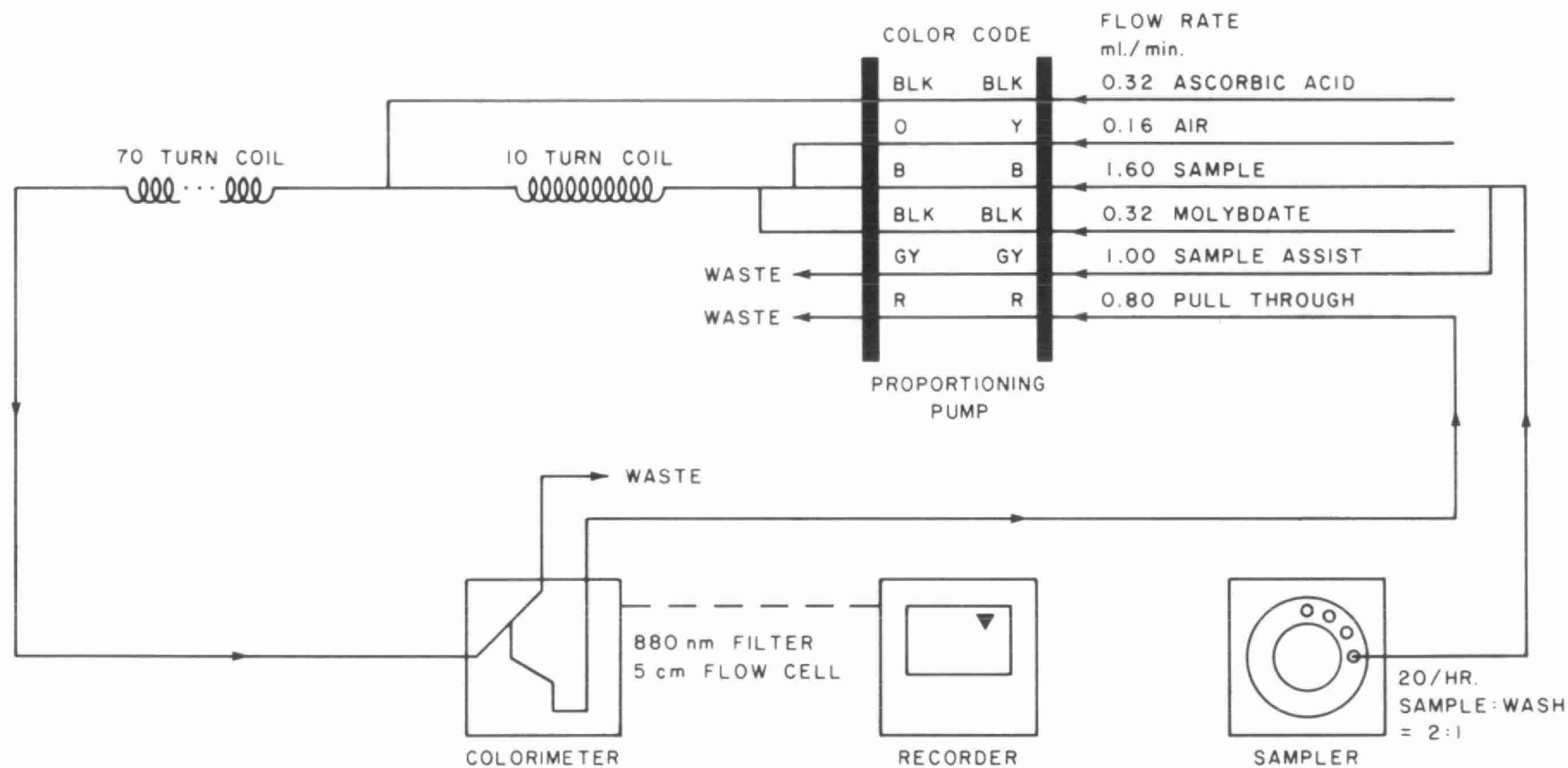


FIGURE 6 — AUTOANALYZER AAII SYSTEM FOR FILTERED TOTAL PHOSPHORUS DETERMINATIONS IN SURFACE WATERS

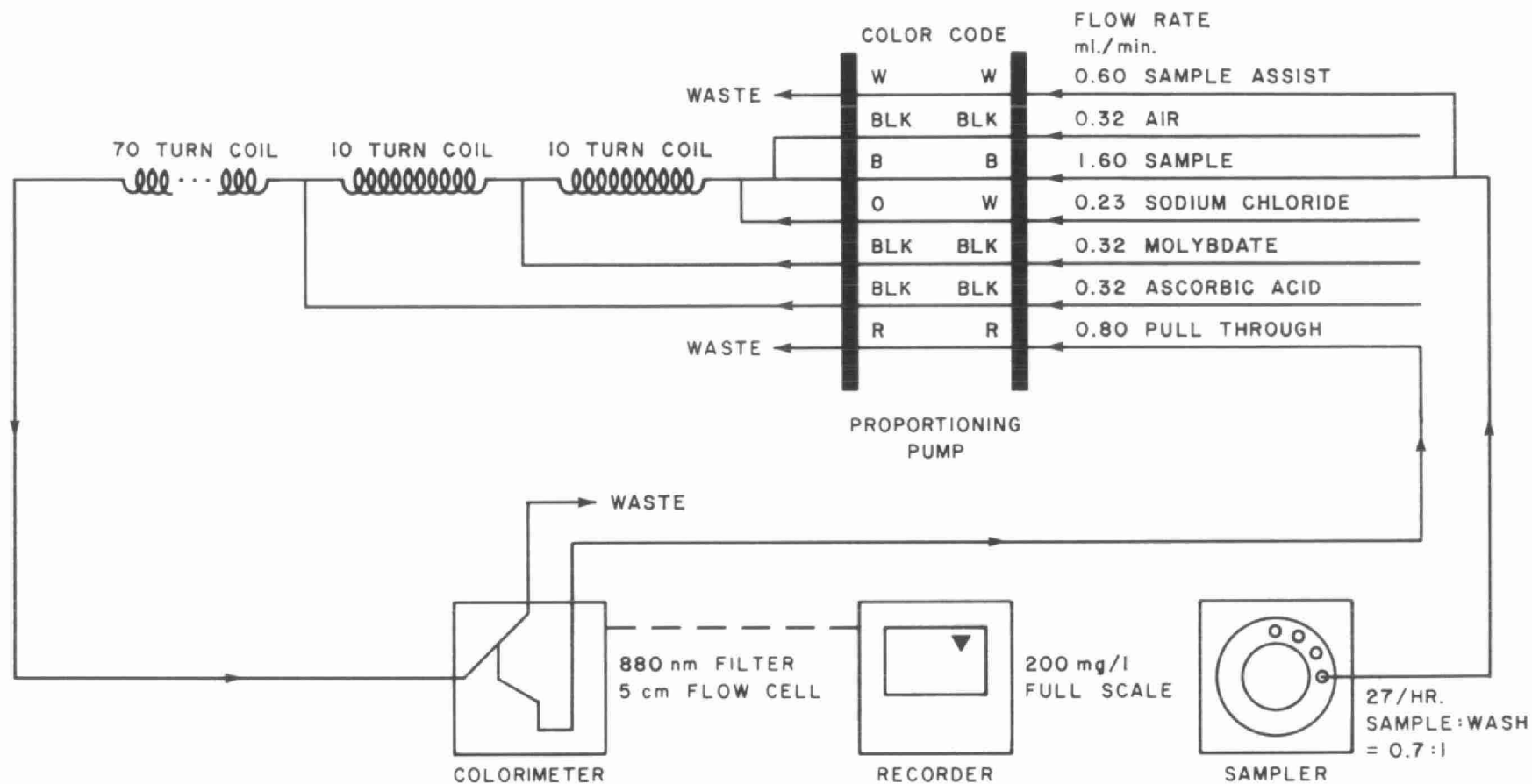


FIGURE 7 — AUTOANALYZER AAII SYSTEM FOR TOTAL PHOSPHORUS DETERMINATION IN SURFACE WATERS



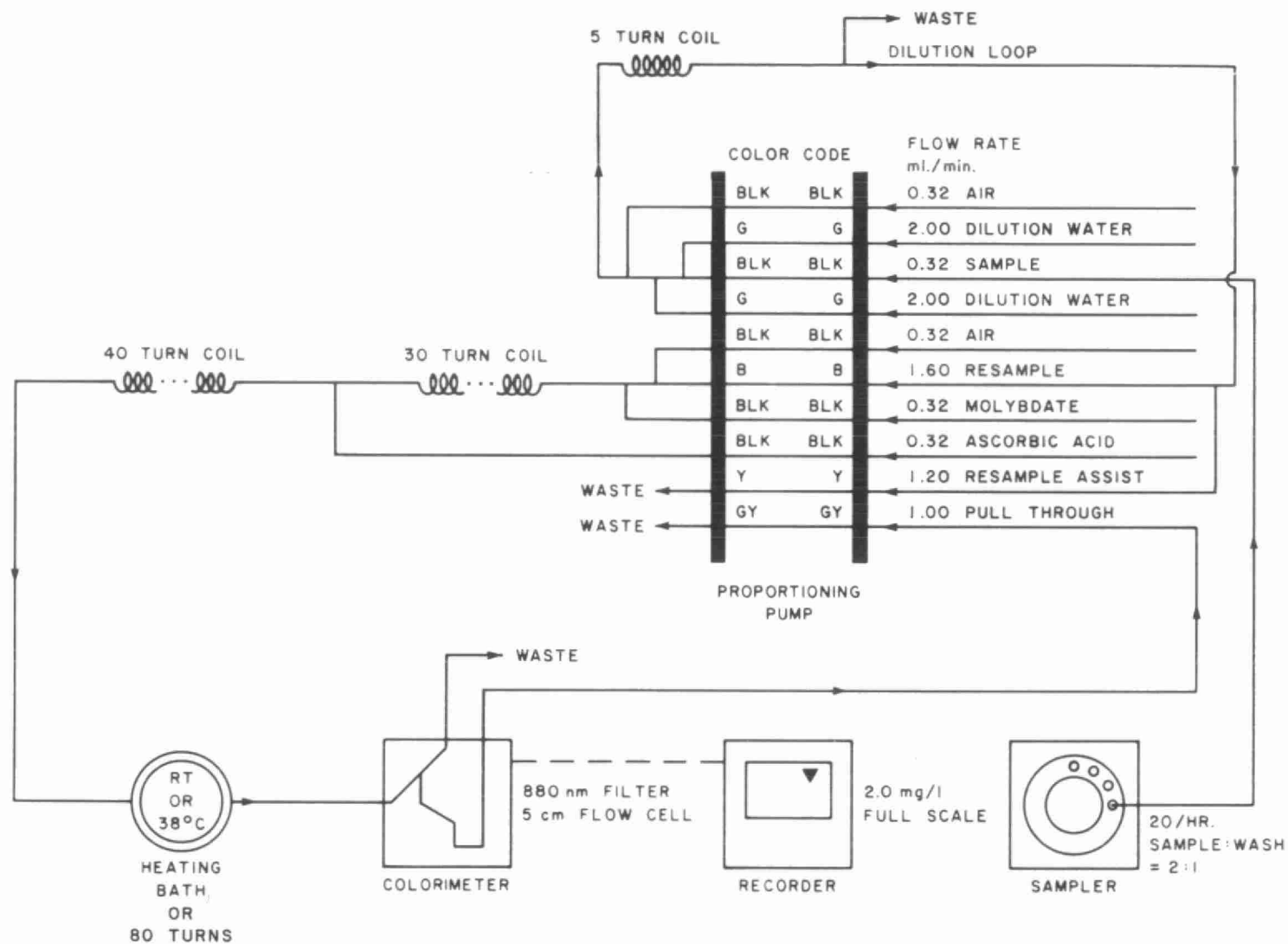


FIGURE 8 — AUTOANALYZER AAII SYSTEM FOR TOTAL PHOSPHORUS DETERMINATIONS IN SEWAGE AND INDUSTRIAL WASTE

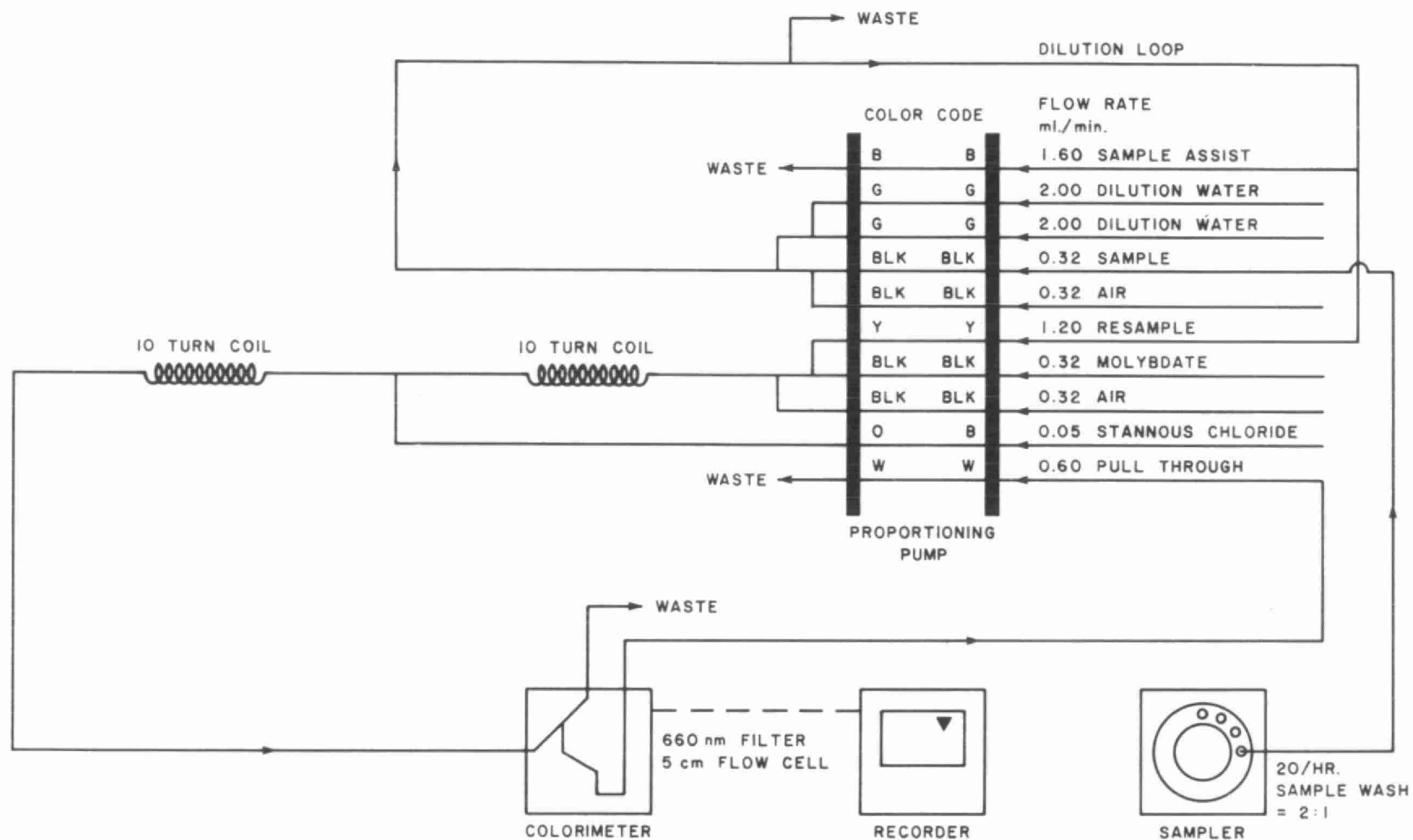


FIGURE 9 — AUTOANALYZER II SYSTEM FOR TOTAL PHOSPHORUS DETERMINATION  
IN SOILS AND VEGETATION

## THE DETERMINATION OF POLYNUCLEAR AROMATIC HYDROCARBONS

The occurrence of polynuclear aromatic hydrocarbons (PAH) as pollutants in air is widespread. Hydrocarbons, such as benzo(a)pyrene, have been found to be potent carcinogens. However, no criteria or standards have been established for these compounds in Ontario.

Polynuclear aromatic hydrocarbons are formed during heating, burning, incineration and other combustion processes of many types of carbonaceous materials. Coal, coke, petroleum based fuels (gasoline and heating oils) and aromatic compounds in general are particularly prone to the production of these compounds under conditions of insufficient amounts of oxygen and/or low combustion temperatures.

In the presence of air, light and/or heat, some PAH compounds are reported to undergo chemical changes such as dimerization, photodegradation, oxidation, etc., particularly when dissolved in solvents or adsorbed on active surfaces.

### Sample Handling and Preservation

#### Air Particulate

Since polynuclear aromatic hydrocarbons exhibit high melting points and relatively low vapor pressures, these materials are present in air in airborne particulate and may be collected on pre-extracted High Volume glass fibre or cellulose filters. The exposed filters should be kept in protective envelopes and stored in a cool and dry atmosphere. The filters are extracted with cyclohexane. Once the extraction is started, the subsequent steps of the procedure should be carried out with as little delay as possible. Overnight storage of extracts, column effluents and sample concentrates should be carried out in closed containers in the dark under refrigeration.

### Selection of Method

Prior to 1977, liquid chromatographic separation at atmospheric pressure and fluorometry were used in the Ministry laboratories for the determination of two PAH compounds, namely benzo(a)pyrene (BaP) and benzo(k)fluoranthene (BkF) (8.1). A high pressure liquid-chromatographic fluorometric method is now used to determine the following PAH compounds: fluoranthene; perylene; benzo(k)fluoranthene (BkF); benzo(a)pyrene (BaP); benzo(g,h,i)perylene (BghiP) and ortho-phenylene pyrene (OPP). This method, also called "high performance liquid chromatography" (HPLC) is a particularly advantageous technique for the separation of these high-molecular hydrocarbon compounds. It is fast; thermally labile compounds are not affected since it operates at ambient temperature; the analysis is nondestructive; it is possible to collect fractions for further analysis by other methods; fluorescence and other detectors can be used by which PAH compounds can be determined at low detection limits and with high specificity; and reverse-phase column packings are suitable for separating isomeric PAH compounds which cannot be separated by other means, including gas chromatography (8.2).

For the selective detection and quantification of PAH compounds separated by HPLC from different types of environmental samples, a fluorescence detector in conjunction with an ultraviolet detector can be used. This method also may be used to measure pyrene, benzo(a)anthracene, chrysene, benzo(e)pyrene and dibenzo(a,h)anthracene, although presently, these compounds are not routinely determined.

## POLYNUCLEAR AROMATIC HYDROCARBONS

### HPLC - Fluorometric Method

#### SUMMARY

<b>Matrix.</b>	This method is used for the determination of PAH in airborne particulate matter.
<b>Substance determined.</b>	Fluoranthene, perylene, benzo(k)fluoranthene, benzo(a)pyrene, benzo(g,h,i)perylene and ortho-phenylene pyrene are determined.
<b>Interpretation of results.</b>	Results are reported in $\mu\text{g}/1000\text{ m}^3$ ambient air and in $\mu\text{g}/\text{g}$ total suspended particulate (TSP). These results are used to monitor PAH concentration levels in air.
<b>Principle of method.</b>	Following extraction of the High Volume filters with cyclohexane, the PAH compounds are separated by high pressure liquid chromatography using an acetonitrile - water eluent. The PAH compounds are identified by their retention times and specific fluorescence responses as compared with those of standards. Analyses are carried out at fixed wavelengths. The fluorescence wavelength, $\lambda$ (excitation) = 360 nm and $\lambda$ (emission) > 470 nm.
<b>Time required for analysis.</b>	<p>Sample preparation: A series of 12 filters are usually extracted simultaneously over a period of 14 hours. Evaporation of these extracts and further sample preparation takes an additional 8 hours.</p> <p>Analysis: HPLC separation of PAH compounds from 12 sample extracts, followed by identification and measurement of these PAH compounds by fluorescence may take a total of 3 days.</p>
<b>Range of application.</b>	Concentrations of 0.01 - 1000 $\mu\text{g}/1000\text{ m}^3$ air can be measured. If higher concentrations of PAH are present, the sample is brought into range by dilution.
<b>Standard deviation.</b>	Results of replicate chromatographic analyses by this method should not differ by more than 5% of the mean.
<b>Accuracy.</b>	While the efficiency of the PAH extraction of the filter sample may vary with the emission, the accuracy of the subsequent analysis of the extracted PAH should be within 10% of the mean.
<b>Detection criteria.</b>	0.01 $\mu\text{g}/1000\text{ m}^3$ air.

**Interferences and shortcomings.**

A decrease in PAH concentrations can result from exposure of PAH extracts or solutions to intense light over extended periods of time, or to heat (during the evaporations).

**Minimum volume of sample.**

This depends on PAH concentration in air sampled using Hi-Vol filters or other airborne particulate collecting devices. For low level concentrations of PAH compounds a 1/10 portion of the Hi-Vol filter is considered sufficient for each analytical run.

**Preservation and sample container.**

The exposed Hi-Vol filters should be kept in protective envelopes and stored in a dark, cool, dry area. Under these conditions, significant losses of PAH should not occur even after several months of storage.

**Safety considerations.**

Use surgical gloves when handling Hi-Vol filters, PAH reference compounds, cyclohexane and acetonitrile. Avoid inhaling the dust or vapors from these materials.

Cyclohexane and acetonitrile are highly flammable. Do not use near an open flame. A fire extinguisher should be available.

## POLYNUCLEAR AROMATIC HYDROCARBONS

### HPLC - Fluorometric Method

#### 1. Introduction

The determination of PAH compounds in airborne particulate, inclusive of sample preparation occurs in 3 stages: 1) the Soxhlet extraction of aliquots of the glass fibre filter samples with cyclohexane followed by the flash evaporation of the extract to a reduced volume; 2) the high-pressure chromatographic separation of PAH compounds, accomplished on a prepacked stainless steel column (Vydac 201 TP reverse phase column); and 3) the detection and quantification of PAH compounds, as they are separated, by a fluorescence detector. The fluorescence wavelengths used are  $\lambda$  (excitation) = 360 nm and  $\lambda$  (emission) > 470 nm.

#### 2. Interferences and Shortcomings

There are no compounds in airborne particulate that are known to interfere with the determination of the six PAH compounds under investigation.

#### 3. Apparatus

- 3.1. Soxhlet extraction assembly including flat bottom flasks, 125 ml capacity, and thimbles, Whatman (free of fluorescence) 25 x 80 mm.
- 3.2. High-pressure chromatographic pump, Varian Model 8500.
- 3.3. Reverse phase stainless steel column, Vydac 201 TP.
- 3.4. Sample loop, 50  $\mu$ l capacity.
- 3.5. Injection valve, 6-port, stainless steel.
- 3.6. Fluorescence detector, Varian Fluorichrom, with excitation band filters 7-54 and 7-60 (for  $\lambda$  = 360 nm) and emission cut-off filters 4-76 and 3-71 (for  $\lambda$  > 470 nm).
- 3.7. Strip-chart recorder.
- 3.8. Syringes, 1 and 5 ml capacities, for HPLC use.
- 3.9. Centrifuge, with 15 ml graduated conical centrifuge tubes.
- 3.10. Volumetric flasks, 5 and 50 ml capacity.
- 3.11. Graduated cylinder, 10 and 100 ml capacity.
- 3.2. Cahn micro-balance.

#### 4. Reagents

- 4.1. Cyclohexane ( $\text{CH}_2(\text{CH}_2)_4\text{CH}_2$ ), nanograde, distilled in glass.
- 4.2. Acetonitrile ( $\text{CH}_3\text{CN}$ ), spectrograde.
- 4.3. Acetone ( $\text{CH}_3\text{COCH}_3$ ), spectrograde.
- 4.4. Fluoranthene, for standards.
- 4.5. Perylene, for standards.
- 4.6. Benzo(k)fluoranthene, for standards.
- 4.7. Benzo(a)pyrene, for standards.
- 4.8. Benzo(ghi)pyrene, for standards.
- 4.9. Ortho-phenylene pyrene (or indeno(1,2,3,c,d)pyrene), for standards.

#### 4.10. Standard Stock Solutions

Weigh accurately to 3 decimal places, approximately 0.2 mg fluoranthene on a Cahn microbalance and transfer into a 50 ml volumetric flask. Dissolve and make up to volume with pure acetonitrile. Mix until homogenous solution is attained to give the fluoranthene standard stock solution.

Standard stock solutions of perylene, benzo(k)fluoranthene (BkF), benzo(a)pyrene (BaP), benzo (ghi) perylene (BgHiP) and ortho-phenylene pyrene (OPP) are prepared in the identical way but with the exception that 2 mg (accurately to 3 decimal places) instead of 0.2 mg of benzo(ghi)perylene are used due to the lower detector sensitivity for this compound.

#### 4.11. Standard Working Solution

Using a precision syringe, transfer 2 ml of each standard stock solution into a 50 ml volumetric flask (12 ml total stock solution in flask). Make up to volume with aqueous 75% acetonitrile. The concentrations of the PAH compounds in this solution are now within 100-200 ng/ml with the exception of benzo(ghi)perylene which ranges from 1000-2000 ng/ml.

#### 5. Procedure

##### 5.1. Extraction of Hi-Vol Filters

- 5.1.1. Cut a 1/10 portion of the Hi-Vol filter (17.78 cm x 22.86 cm) with a standard die and transfer to a Soxhlet thimble.
- 5.1.2. Using 80-85 ml cyclohexane proceed with the Soxhlet extraction for 14 hours at rapid reflux.
- 5.1.3. Evaporate the extract to about 3 ml volume under reduced pressure, using a bath temperature of 40-45°C.



- 5.1.4. Transfer the concentrate to a 10 ml graduated cylinder, rinse the flask with a few drops of cyclohexane for quantitative transfer and make up to 4 ml with cyclohexane.
- 5.1.5. Transfer half of this volume (2 ml) into a graduated 15 ml centrifuge tube and blow down this volume with nitrogen gas to dryness, at room temperature, and make to 1 ml with aqueous 75% acetonitrile. Centrifuge prior to analysis. The remaining half of the extract is retained for duplicate analysis, if necessary.

## 5.2. PAH Separation by HPLC

- 5.2.1. Using a 1 ml precision syringe, inject a 0.1 ml volume of the final acetonitrile extract into a 50  $\mu$ l sample loop.
- 5.2.2. Inject the sample from the sample loop onto the Vydac 201 TP reverse phase column using 75% acetonitrile in water as the mobile phase. The flow rate is 60 ml/hr and a fluorescence detector is used for the analysis of PAH compounds in the eluent from the column.
- 5.2.3. Using a chart speed of 0.5 cm/min, record the detector responses measure the peak heights obtained for the PAH compounds.

## 5.3. PAH Determination

- 5.3.1. Inject and run a standard before and after each series of samples (a maximum of 5 samples per series is suggested) to obtain an average response factor between the beginning and the end of each series of samples.
- 5.3.2. Compare the retention times of the standard with the retention times of the various compounds in the sample and identify the sample peaks. PAH compounds are measured by peaks produced in the following order: fluoranthene; perylene; benzo(k)fluoranthene; benzo(a)pyrene; benzo(g,h,i,)perylene; ortho-phenylene pyrene.
- 5.3.3. Measure peak heights using a baseline drawn across the base of the peaks of interest. The peak heights determine PAH concentrations in the injected sample by correlation with the corresponding peak heights of the standard..

## 6. Calculation and Reporting

- 6.1. Calculate the PAH concentration in air according to the equations shown below and report in  $\mu$ g/1000 m<sup>3</sup> air.

$$\mu\text{g}/1000\text{ m}^3\text{ air} = \frac{a \times b \times c}{d}$$

Where:

- a = filter factor (see 6.2.)
- b = average response factor (see 6.3.)
- c = peak height (cm)<sub>3</sub> x attenuation
- d = volume of air (m<sup>3</sup>) drawn through the filter

6.2. Calculate the filter factor as follows:

$$\text{Filter factor} = \frac{2}{\text{portion of Hi-Vol filter extracted}}$$

6.3. Calculate the average response factor as follows:

$$\text{Average Response factor} = \frac{c}{p \times a}$$

Where:

c = concentration (ng/ml) of standard  
p = peak height, average of standard  
a = attenuation

## 7. Precision and Accuracy

Results of replicate chromatographic analyses should not differ by more than 5% of the mean. While the efficiency of the PAH extraction of the filter may vary with the adsorbed particulates, the accuracy of the subsequent analysis of the extracted PAH should be within 10% of the mean.

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## THE DETERMINATION OF POTASSIUM

Potassium is the seventh most abundant element on earth, comprising 2.4% of the earth's crust. It is an essential nutrient for all life forms, especially plants, and is included as a major component of fertilizers. Despite the substantial amounts of potassium dispersed over the land as fertilizer, it seldom appears in any great quantity in Ontario waters. Many soils have the ability to fix potassium and prevent its migration through leaching.

Potassium concentrations rarely exceed 20 mg/l in natural waters. It is normally found in association with sodium, and in only a few cases (usually indicating an artificial source) will it ever exceed sodium in concentration. Potassium is non-toxic and represents no known health hazard; hence no drinking water standard has been established.

### Sample Handling and Preservation

#### Water, Sewages and Industrial Wastes

Plastic containers are preferred and required for precipitation samples since soft glass, and to a lesser extent Pyrex, release potassium ions to the sample (usually less than 0.05 mg/l), particularly at a high pH. When near neutral samples with potassium concentrations sufficiently large to render this container effect negligible are to be collected, glass bottles may be used.

The usual precaution of rinsing the container several times with sample should be observed; no preservative is required.

For soil sampling procedures see The Determination of Trace Metals by Atomic Spectroscopy.

### Selection of Method

Method A is an automated atomic absorption technique which employs five ranges, two each for surface waters and drinking water and one for precipitation samples. Slightly different instrumentation is used for each different type of sample. The regional laboratories also use this method although ranges and consequently standard and quality control solutions concentrations may vary. Capability for in-line addition of ionization suppressing reagent and diluent is included in the automated sampling system. An X-ray fluorescence method is used on vegetation samples and is described in the Determination of Trace Metals by Atomic Spectroscopy. An atomic absorption method is used for soil samples and an ICP method is used for soils and sediments. These methods are described in the Determination of Trace Metals by Atomic Spectroscopy.

## POTASSIUM

### Automated Atomic Absorption Method A

#### SUMMARY

<b>Matrix.</b>	This method is used on surface waters, precipitation samples drinking waters, sewage and industrial wastes
<b>Substance determined.</b>	Potassium ion ( $K^+$ ).
<b>Interpretation of results.</b>	The results are reported as mg/l K.
<b>Principle of method.</b>	An automated atomic absorption method is used to measure the concentration of potassium ions. The sample is diluted with an ionization suppressant, cesium chloride, prior to aspiration into the burner flame.
<b>Time required for analysis.</b>	A single determination requires 15 minutes; however, by suitable batch arrangement, 150 samples a day may be analyzed.
<b>Range of application.</b>	Surface waters: a) 0.05 - 5.0 mg/l b) 5.0 - 10.0 mg/l Precipitation: 0.01 - 1.00 mg/l Drinking water: 0.1 - 10 mg/l 10 - 40 mg/l
<b>Standard deviation.</b>	Relative standard deviations are 1.6% for low range surface waters, 2.5% for high range surface waters, 1.9% for precipitation samples and 0.7% for drinking waters.
<b>Accuracy.</b>	Calibration is controlled by 2 independently prepared long term standards, QC-A and QC-B in such a way that $(A + B)$ and $(A - B)$ do not vary by more than 3 standard deviations from their long-term means. These control limits are: 0.102 mg/l for low range surface waters, 0.42 mg/l for high range surface waters; 0.021 for precipitation samples, 0.30 for low range drinking waters and 0.7 mg/l for high range drinking waters.
<b>Detection criteria.</b>	0.066 for low range surface waters; 0.107 for high range surface waters; 0.021 for precipitation samples and 0.061 for drinking water samples.

**Interferences and shortcomings.**

Partial ionization of the potassium atoms in the flame yields correspondingly low results. Automatic addition of cesium chloride solution to achieve a final cesium level of 500 mg/l, (250 mg/l for precipitation) is employed to control this problem. Careful maintenance of stable aspiration and burner conditions is also important.

**Minimum volume of sample.**

50 ml for surface and drinking water samples, 10 ml for precipitation samples.

**Preservation and sample container.**

No special preservation measures are required. Use of plastic sampling containers is advised to eliminate potassium leaching problems encountered with glass bottles; for the case in which expected sample concentration is sufficiently high so as not to be significantly influenced by small additions from the glass (0.05 mg/l even at a high pH), glass sample containers are acceptable.

**Safety considerations.**

The possibility of burner flash-back or explosion is always present when using flame atomic absorption apparatus. The manufacturer's instructions for burner ignition, use, and shut-down must always be rigorously followed, and the waste trap filled with water at all times. Standard safety procedures should be employed when working with compressed gas cylinders.

## POTASSIUM

### Automated Atomic Absorption Method A

#### 1. Introduction

The sample under test, automatically diluted with an ionization suppressant, is aspirated as a fine mist into an air-acetylene flame. Light emitted from a hollow cathode lamp at a characteristic wavelength for potassium, is directed through the flame into a monochromator and onto a detector. Potassium atoms, heated in the flame, absorb this light and the detector measures the decreased intensity of the resulting beam. The amount of light absorbed is directly proportional to the concentration of potassium in the sample, and is recorded on a strip-chart recorder as a series of peaks. Calibration, at the low levels of dilution made possible by the sensitivity of the AAS instrument, is completely linear.

In most cases, unfiltered samples may be used. However, when visible suspended matter is present, filtration through glass fibre filter paper is necessary.

#### 2. Interferences and Shortcomings

Partial ionization of potassium atoms in the flame results in a decrease in absorbance, and low results. The degree of ionization is a function of temperature; hence for reproducibility, stable aspiration and flame conditions must be maintained for both standards and samples throughout an analytical run. Potassium ionization is suppressed by using cesium chloride, which is automatically added to the sample. A final cesium level of 500 mg/l (250 mg/l for precipitation) at the burner is employed.

Partial clogging of the burner nebulizer and consequent reduction in aspiration may result from processing samples containing large amounts of suspended solids; pre-filtration of samples is advisable in this case.

#### 3. Apparatus

- 3.1. Atomic absorption spectrophotometer, a Varian AA275 is used for precipitation samples, a Pye Unicam SP 1900 for surface waters and a Varian AA5 for drinking water samples.
- 3.2. Sample changer. A Gilson sampler is used for surface waters and drinking waters and a Technicon sampler is used for precipitation samples.
- 3.3. Proportioning pump and manifold pump tubing and glassware as shown in Figures 1 to 4.
- 3.4. Strip chart recorder.
- 3.5. Sampling tubes to fit the sampler used. Disposable plastic specimen tubes are used for precipitation samples.

A routine burner maintenance program is recommended which ensures clean burner head and mixing chamber conditions, clean gas and drainage tubes, and a nebulizer free of blockage. Such a program is not only necessary from a safety standpoint, but will also provide efficient, accurate operation of the AAS.

#### 4. Reagents

4.1. Potassium chloride (KCl), reagent grade crystals.

4.2. Cesium chloride (CsCl), reagent grade crystals.

#### Surface Waters

##### 4.3. Potassium Stock Solution (4,000 mg/l)

In a 500 ml volumetric flask, dissolve 3.8134 g KCl (oven dried and cooled in a desiccator) in distilled, deionized water and dilute to the mark.

**NOTE:** If calcium, magnesium, and sodium are also to be measured see individual methods for preparation of their respective stock solutions.

##### 4.4. Combined Intermediate Solution (low range 0.05 - 5.0 mg/l range)

In a 1 liter volumetric flask, pipet 25 ml potassium stock solution, 5 ml sodium stock solution, 10 ml calcium stock solution and 5 ml magnesium stock solution. Dilute to the mark with distilled, deionized water. This solution contains 100 mg/l potassium, 200 mg/l sodium, 400 mg/l calcium and 100 mg/l magnesium.

##### 4.5. Combined Intermediate Solution (high range 0.1 - 10.0 mg/l range)

In a 1 liter volumetric flask, dilute a 50 ml aliquot of each of potassium, sodium, calcium and magnesium stock solutions to the mark with distilled, deionized water. This solution contains 200 mg/l potassium, 2000 mg/l sodium, 2000 mg/l calcium and 1000 mg/l magnesium.

##### 4.6. Combined Calibration Standards (low range 0.05 - 5.0 mg/l)

In volumetric flasks, dilute the following aliquots of combined intermediate solution (reagent 4.4) to 1000 ml with distilled, deionized water to give combined working standards and allow the determination of four elements.

Aliquot	Potassium	Sodium	Calcium	Magnesium
50 ml	5	10	20	5
40 ml	4	8	16	4
30 ml	3	6	12	3
20 ml	2	4	8	2
10 ml	1	2	4	1
5 ml	0.5	1	2	0.5

#### 4.7. Combined Calibration Standards (high range 5 - 10 mg/l)

In volumetric flasks, dilute the following aliquots of combined intermediate solution (reagent 4.5) to 1000 ml with distilled, deionized water to give combined working standards.

Aliquot	Potassium	Sodium	Calcium	Magnesium
50 ml	10	100	100	50
40 ml	8	80	80	40
30 ml	6	60	60	30
20 ml	4	40	40	20
10 ml	2	20	20	10
5 ml	1	10	10	5

#### 4.8. Quality Control Stock Solution (low range, 72 mg/l K)

In a 1 liter volumetric flask and using a different batch of potassium chloride than the one used for the preparation of reagent 4.3, dissolve 0.1373 g potassium chloride (oven dried and cooled in a desiccator) in distilled, deionized water and dilute to 1 liter.

#### 4.9. Quality Control Working Solutions (low range)

QC-A: In a 1 liter volumetric flask dilute 50 ml quality control stock solution (reagent 4.8) to 1 liter with distilled, deionized water, to give a solution which is 72% of scale.

QC-B: In a 1 liter volumetric flask dilute 20 ml quality control stock solution (reagent 4.9) to 1 liter with distilled, deionized water, to give a solution which is 28.8% of scale.

#### 4.10. Quality Control Stock Solution (high range, 144 mg/l K)

In a 1 liter volumetric flask, and using a different batch of potassium chloride than the one used for the preparation of reagent 4.3, dissolve 0.2746 g potassium chloride (oven dried and cooled in a desiccator) in distilled, deionized water and dilute to 1 liter.

#### 4.11. Quality Control Working Solutions (high range)

QC-A: In a 1 liter volumetric flask, dilute 50 ml quality control stock solution (reagent 4.10) to 1000 ml with distilled, deionized water to give a solution which is 72% of scale.

QC-B: In a 1 liter volumetric flask dilute 20 ml quality control stock solution (reagent 4.10) to 1000 ml with distilled, deionized water to give a solution which is 28.8% of scale.

**NOTE:** For quality control solutions for calcium, magnesium and sodium see individual methods.

#### 4.12. Cesium Chloride Suppressant

Dissolve 8.18 g cesium chloride in distilled, deionized water and dilute to 4 liters.



## Precipitation Samples

### 4.13. Potassium Stock Solution (1000 mg/l)

In a 1 liter volumetric flask, dissolve 1.9069 g potassium chloride (oven dried and cooled in a desiccator) in distilled, deionized water and dilute to the mark.

**NOTE:** If calcium, magnesium and sodium are to be measured also, see write-up in corresponding methods for instructions pertaining to the preparation of stock solutions for these elements.

### 4.14. Combined Sodium-Potassium Intermediate Stock Solution

In a 1 liter volumetric flask, dilute 20 ml sodium stock solution (see the Determination of Sodium) and 20 ml potassium stock solution to the mark with distilled, deionized water. This solution contains 20 mg/l potassium and 20 mg/l sodium.

**NOTE:** If calcium and magnesium are also to be measured a combined calcium-magnesium intermediate solution is also prepared by diluting 10 ml magnesium and 10 ml calcium stock solution to 1000 ml with distilled, deionized water.

### 4.15. Combined Sodium-Potassium Working Standards

Into a 1 liter volumetric flask, pipette 5.00, 10.00, 20.00, 30.00, 40.00 and 50.00 ml combined sodium-potassium intermediate solution and dilute each to 1 liter with distilled, deionized water. This gives working standards with potassium and sodium concentrations of 0.10, 0.20, 0.40, 0.60, 0.80 and 1.00 mg/l respectively.

**NOTE:** For calcium, magnesium working standards see the Determination of Calcium (or Magnesium).

### 4.16. Quality Control Stock Solution (1000 mg/l)

Prepare a potassium quality control stock solution which is identical to reagent 4.13 but using a different batch of potassium chloride.

**NOTE:** For calcium and magnesium quality control stock solutions see the Determination of Calcium (or Magnesium).

### 4.17. Combined Sodium-Potassium Quality Control Intermediate Solution

In a 1 liter volumetric flask, dilute 20 ml sodium quality control stock solution (see the Determination of Sodium) and 20 ml potassium quality control stock solution to the mark with distilled, deionized water. This solution contains 20 mg/l potassium and 20 mg/l sodium.

**NOTE:** If calcium and magnesium are also to be determined a combined calcium-magnesium quality control intermediate solution is also prepared. See the Determination of Calcium (or Magnesium).

### 4.18. Quality Control Working Solutions

QC-A: In a 1 liter volumetric flask, dilute 30 ml combined sodium-potassium quality control intermediate solution to the mark with distilled, deionized water to give a solution with a sodium concentration of 0.60 mg/l and a potassium concentration of 0.60 mg/l.

QC-B: In a 1 liter volumetric flask, dilute 5 ml combined sodium-potassium quality control intermediate solution to the mark with distilled, deionized water to give a solution with a sodium concentration of 0.10 mg/l and a potassium concentration of 0.10 mg/l.

**NOTE:** For quality control working solutions for calcium and magnesium see the Determination of Calcium and the Determination of Magnesium.

#### **4.19. Cesium Chloride Suppressant (2500 mg/l Cs)**

Dissolve 3.166 g cesium chloride in distilled, deionized water and dilute to 1 liter, for a concentration 2500 mg/l CsCl.

### **Drinking Water Samples**

#### **4.20. Combined Stock Solution (4000 mg/l potassium)**

Prepare a combined stock solution by dissolving, in a 1 liter volumetric flask, 50.8424 g sodium chloride (oven dried and cooled in a desiccator); 7.6273 g potassium chloride (oven dried and cooled in a desiccator); 49.447 g calcium carbonate (oven dried, cooled in a desiccator and dissolved in 1:1 hydrochloric acid) and 8.000 g magnesium ribbon (cleaned with hydrochloric acid and dissolved in 1:1 hydrochloric acid). Dilute to the mark with distilled water. This solution has the following concentrations: 20,000 mg/l sodium, 4,000 mg/l potassium, 20,000 mg/l calcium, and 8000 mg/l magnesium.

#### **4.21. Combined Intermediate Solution (low range 0.1 - 10 mg/l).**

In a 1 liter volumetric flask, dilute 25.0 ml combined stock solution (reagent 4.20) to the mark with distilled water. This gives a concentration of 500 mg/l sodium, 100 mg/l potassium 500 mg/l calcium, and 200 mg/l magnesium.

#### **4.22. Combined Intermediate Solution (high range 10 - 40 mg/l)**

In a 1 liter volumetric flask, dilute 100 ml combined stock solution (reagent 4.20) to the mark with distilled water. This gives a concentration of 2000 mg/l sodium; 400 mg/l potassium; 2000 mg/l calcium and 800 mg/l magnesium.

#### **4.23. Potassium Working Solutions**

In a 1 liter volumetric flask dilute: 10.0 ml of combined intermediate solution (low range, reagent 4.21) to the mark with distilled water to give a 10% (1 mg/l K) low range standard.

In a 1 liter volumetric flasks dilute: 100 ml of combined intermediate solution (low range, reagent 4.21) to the mark with distilled water to give a 100% (10 mg/l K) low range standard and 100 ml combined intermediate solution (high range, reagent 4.22) to 1 liter with distilled water to give a 100% (40 mg/l K) high range standard. This gives 3 working standards. The 10 mg/l K standard is used for both the high and low ranges.

#### **4.24. Combined Quality Control Stock Solution**

In a 250 ml volumetric flask, dissolve 1.6524 g sodium chloride (oven dried and cooled in a desiccator); 0.2478 g potassium chloride (oven dried and cooled in a desiccator) in distilled water. Add 0.2600 g magnesium ribbon (cleaned and dissolved in 1:1 v/v hydrochloric acid) and 1.6232 g calcium carbonate (oven dried, cooled in a desiccator) and dissolved in 1:1 v/v hydrochloric acid. Dilute to the mark with distilled water. Use a different batch of chemicals than those used to prepare reagent 4.21.

#### 4.25. Combined Quality Control Working Solutions

- QC-A: In a volumetric flask, dilute 100 ml combined quality control stock solution to 2 liters with distilled water. This solution contains: 26.0 mg/l potassium, 130 mg/l calcium; 52.0 mg/l magnesium and 130 mg/l sodium.
- QC-B: In a volumetric flask, dilute 25 ml combined quality control stock solution to 2 liters with distilled water. This solution contains 6.5 mg/l potassium; 32.5 mg/l calcium; 13 mg/l magnesium and 32.5 mg/l sodium.
- QC-C: In a volumetric flask, dilute 5 ml combined quality control stock solution to 2 liters with distilled water. This solution contains 1.3 mg/l potassium; 6.5 mg/l calcium, 2.6 mg/l magnesium and 1.5 mg/l sodium.

#### 4.26. Cesium Chloride Suppressant

Dissolve 4.05 g cesium chloride in distilled water and dilute to 4 liters.

### 5. Procedure

#### 5.1. Surface Waters

ANALYSES ARE PERFORMED USING AN AIR-ACETYLENE FLAME IN AN IDENTICAL MANNER AS CALCIUM (SEE THE DETERMINATION OF CALCIUM, METHOD B) WITH THE FOLLOWING EXCEPTIONS:

- 5.1.1. With regard to AAS operation, a potassium hollow cathode lamp operated at approximately 50% of the rated lamp current is employed, hence monochromator peaking at the 766.5 nm wavelength for potassium is required.
- 5.1.2. The ionization suppression and dilution reagent used is the CsCl solution designed for the range in use (prepared as outlined previously).
- 5.1.3. The particular pump and manifold design applicable to potassium analysis is given in Figures 1 and 2 for the low and high range respectively.
- 5.1.4. Monochromator slit width should be 0.20 nm.

**NOTE:** THE AAS UNIT MUST NOT BE LEFT UNATTENDED WHILE IT IS IN OPERATION.

DUE TO SLIGHT DIFFERENCES IN ASPIRATION RATES FOR DIFFERENT AAS UNITS, SMALL MODIFICATIONS OF THE MANIFOLD MAY BE NECESSARY TO SATISFY THE BURNER ASPIRATION REQUIREMENT. THIS MODIFICATION IS PARTICULARLY SIMPLE WHEN THE GILSON PROPORTIONING PUMP IS USED SINCE THIS APPARATUS HAS A CONTINUOUSLY VARIABLE PUMPING SPEED. THE FLOW RATE MAY BE INCREASED TO WHATEVER LEVEL IS REQUIRED WHILE MAINTAINING THE OVERALL SAMPLE:SUPPRESSANT:AIR RATIO.

For all other details of the AAS operation, refer to the Determination of Calcium.

## 5.2. Precipitation Samples

THE PROCEDURE FOR POTASSIUM ANALYSIS IS IDENTICAL TO CALCIUM ANALYSIS (SEE THE DETERMINATION OF CALCIUM) WITH THE FOLLOWING EXCEPTIONS.

5.2.1. Set the following controls to the values provided:

lamp current = 5 mA  
slit width = 1.0 nm  
wavelength = 766.5 nm  
burner height = optimum value

For all other details of the AAS operation, the reader is referred to the Determination of Calcium.

5.2.2. The manifold design is given in Figure 3.

## 5.3. Drinking Water

THE PROCEDURE FOR POTASSIUM ANALYSIS IS IDENTICAL TO SODIUM ANALYSIS (SEE THE DETERMINATION OF SODIUM) WITH THE FOLLOWING EXCEPTIONS.

5.3.1. Set the following controls to the values provided:

slit width = 0.3 nm  
wavelength = 766.5 nm

For all other details of the AAS operation, refer to the Determination of Sodium.

5.3.2. The manifold design is given in Figure 4.

## 6. Calculation and Reporting

Comparison of sample absorbance against that of known standards allows calculation of sample concentration, taking into account any dilution factor that may be present. Results are reported as milligram per liter (mg/l) according to the following schedule:

Surface Waters:	Report
<0.05 mg/l	as <0.05 mg/l
0.05 - 9.9 mg/l	to 2 significant figures
>10.0 mg/l	to 3 significant figures

## Precipitation

<0.01

0.01 - 1.00

as <0.01

to nearest 0.01

## Drinking Water

0.1 - 1 mg/l

1 - 9.9 mg/l

10 - 40 mg/l

to 1 significant figure

to 2 significant figures

to 3 significant figures

## 7. Precision and Accuracy

Standard deviations based on within-run duplicate samples are as follows:

Sample type	Range (mg/l)	S <sub>ld</sub>	S <sub>md</sub>	S <sub>hd</sub>
Surface waters	0.05 - 5.00	0.040	0.050	0.061
	0.1 - 10.0	0.065	0.118	0.187
Precipitation	0.01 - 1.00	0.034	0.01	-
Drinking water	0.1 - 40.0	0.037	0.080	0.110

Where:

S<sub>ld</sub> = standard deviation for 0 - 20% of the range

S<sub>md</sub> = standard deviation for 20 - 50% of the range

S<sub>hd</sub> = standard deviation for 50 - 100% of the range

Calibration is controlled by 2 independently prepared quality control standards (QC-A and QC-B) for each range. Control is maintained in such a way that (A + B) and (A - B) do not vary by more than 3 standard deviations from the long-term mean of (A - B). These control limits are 0.102 mg/l for low range surface water; 0.42 mg/l for high range surface waters; 0.021 mg/l for precipitation samples; 0.30 for low range drinking waters and 0.7 mg/l for high range drinking waters.

## 8. Bibliography

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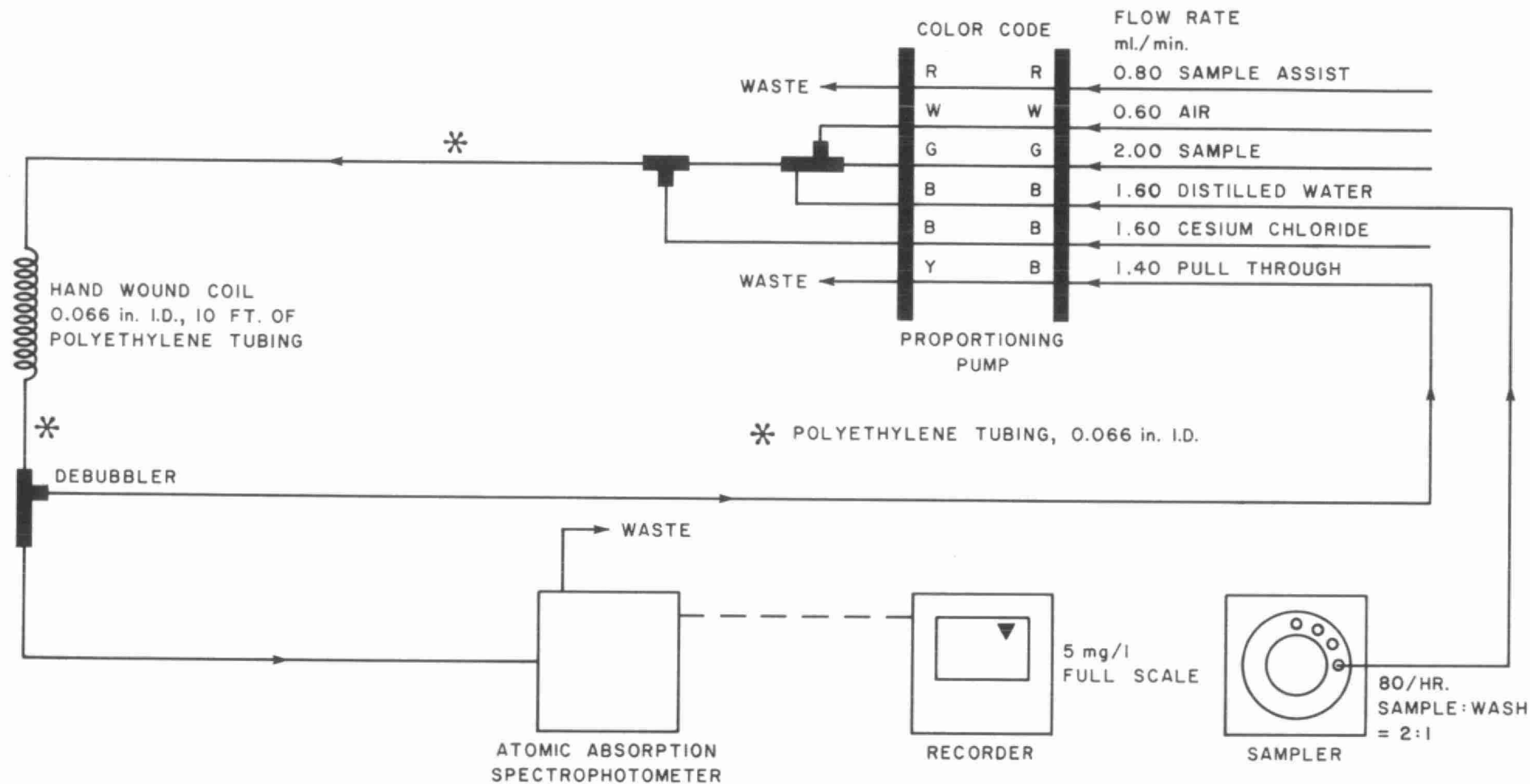


FIGURE 1 — AUTOMATED ATOMIC ABSORPTION MANIFOLD FOR LOW LEVEL POTASSIUM DETERMINATIONS IN SURFACE WATERS

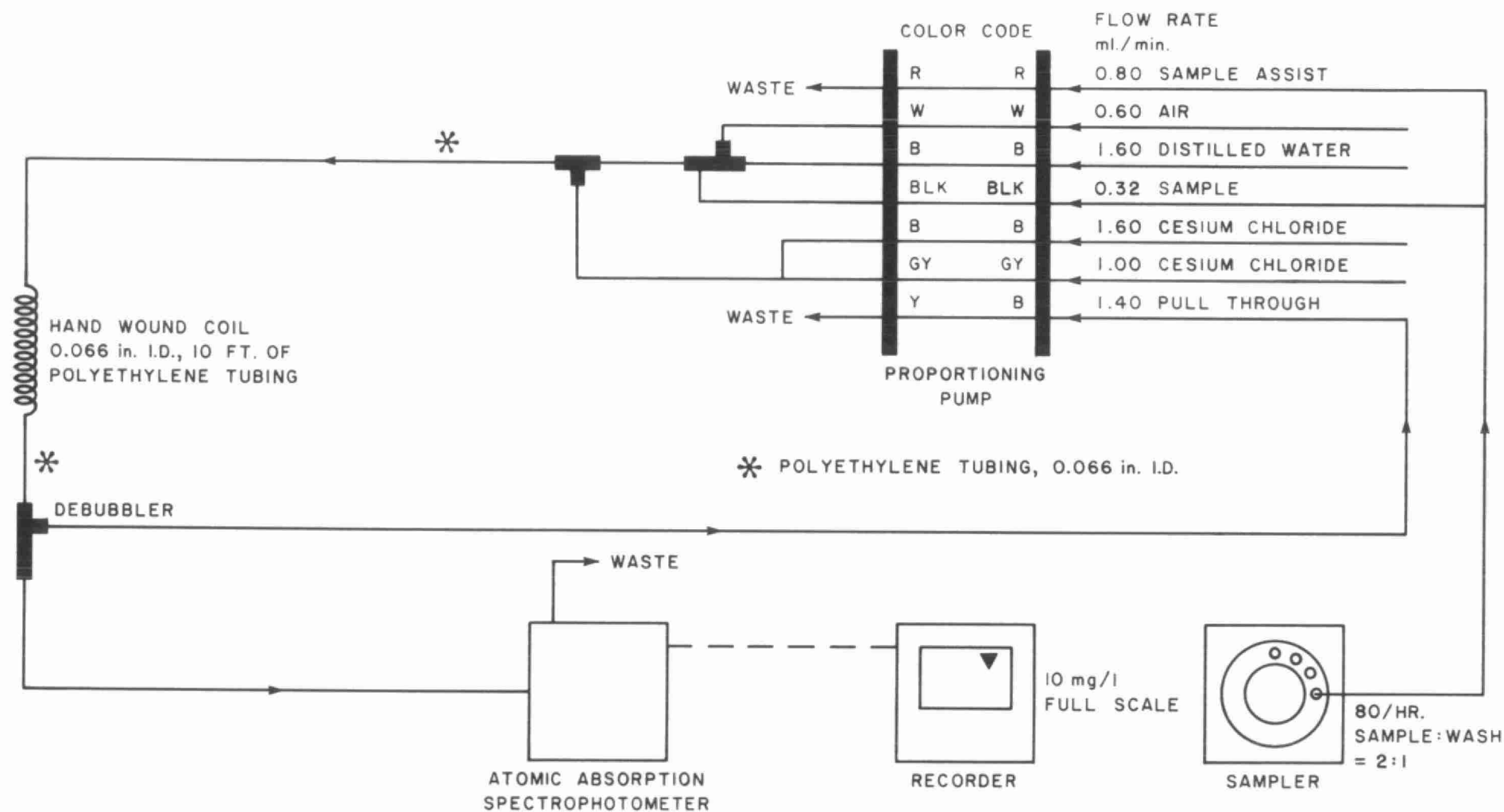


FIGURE 2 — AUTOMATED ATOMIC ABSORPTION MANIFOLD FOR HIGH LEVEL POTASSIUM DETERMINATIONS IN SURFACE WATERS

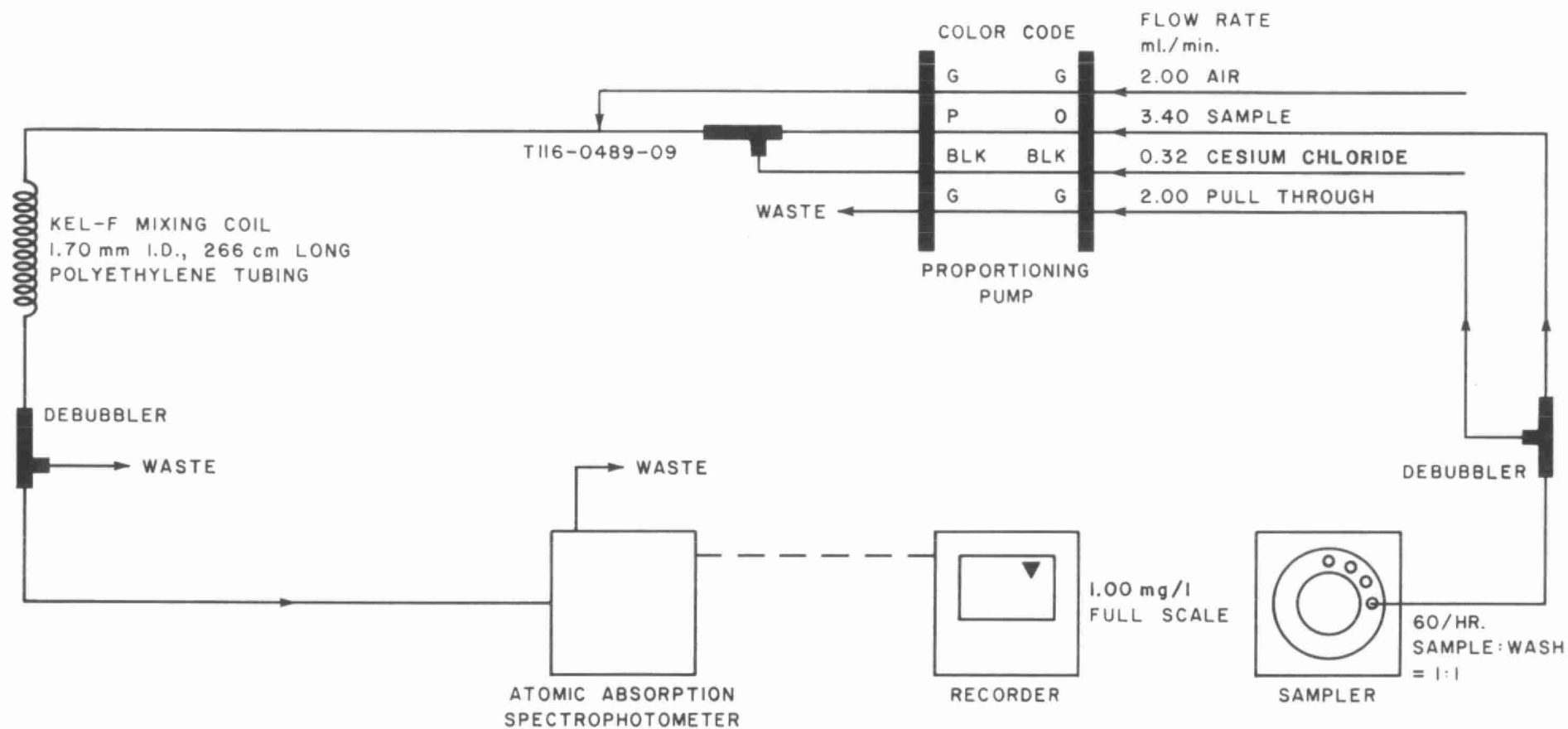


FIGURE 3 — AUTOMATED ATOMIC ABSORPTION MANIFOLD FOR POTASSIUM DETERMINATIONS ON PRECIPITATION SAMPLES



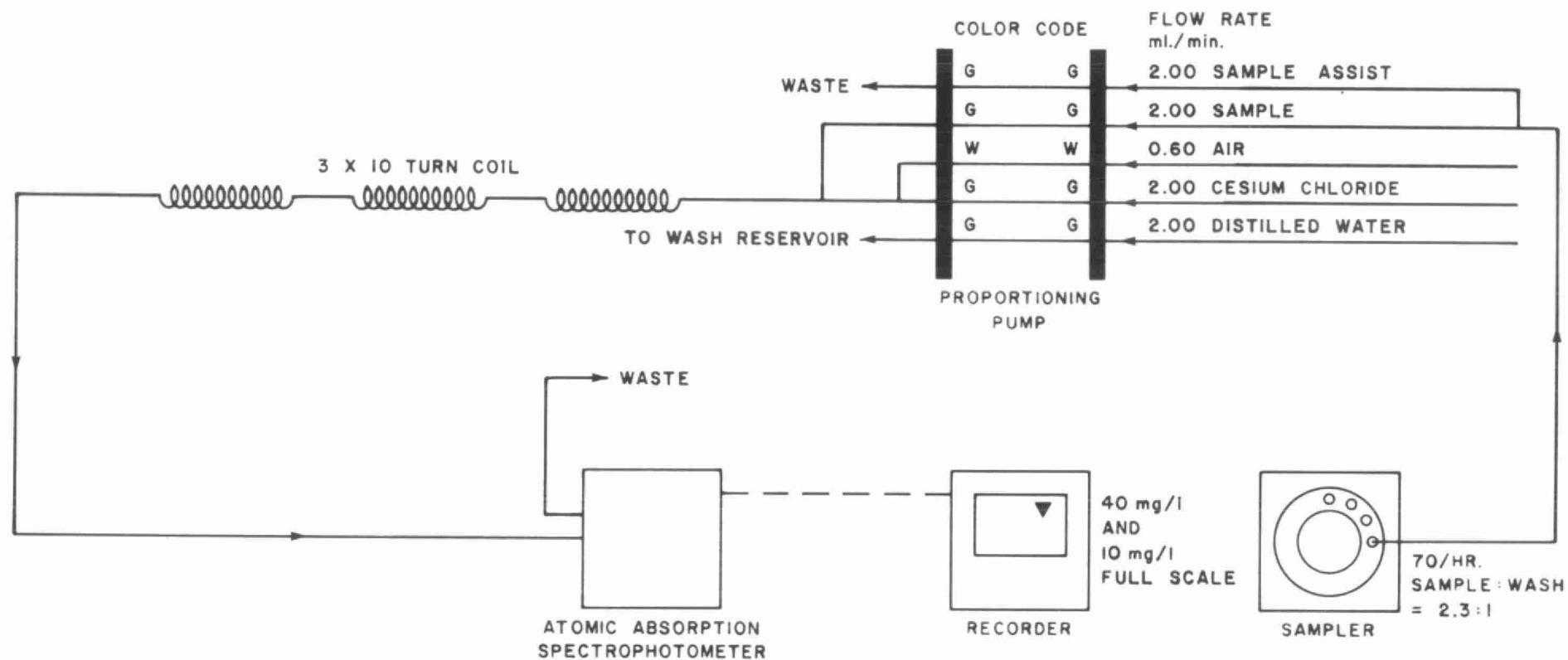


FIGURE 4 — ATOMIC ABSORPTION MANIFOLD FOR POTASSIUM DETERMINATION IN DRINKING WATERS.

## THE DETERMINATION OF SELENIUM

Selenium is usually found in nature as metal selenides in association with sulphide ores. Elemental selenium, basic ferric selenite ( $\text{Fe(OH)SeO}_3$ ), calcium selenate and organo-selenium compounds also occur naturally. Elemental selenium is highly insoluble and requires oxidation to selenite or selenate before appreciable quantities appear in water.

Selenium is used in the manufacture of rectifiers and photoelectric cells, for the coating of stainless steel and copper, as a decolorizer in glass production, in the pigment used in paints, as a supplement to sulphur in vulcanization and a catalyst in the manufacture of nicotinic acid. Selenium oxychloride is used as a solvent and plasticizer.

The role of selenium in human and animal nutrition is not well understood. As an additive to cattle feed it prevents white muscle disease, while excess selenium results in deformed hooves and death. Evidence is mounting to support a connection between selenium and vitamin E in the prevention of gastro-intestinal cancers. Although previous evidence suggested that selenium was carcinogenic, these observations have not been borne out by subsequent data.

In Ontario the permissible criteria for selenium in public water supply is 0.01 mg/l as Se.

### Sample Handling and Preservation

Samples may be collected in either plastic or glass bottles with plastic caps and must be preserved with 1 ml of nitric acid per liter of sample. A minimum of 25 ml is required to conduct a single analysis.

### Selection of Method

Several methods have been evaluated in this laboratory. Gas chromatographic, electro-chemical and fluorometric procedures were all found to be sufficiently accurate, sensitive and interference free. The hydride generation, flameless atomic absorption procedure was chosen because, as well as meeting the above conditions, it was readily automated and thus capable of producing at least twice as many analyses per day as the other procedures.

## SELENIUM

### Hydride Generation - Flameless Atomic Absorption Method

#### SUMMARY

<b>Matrix.</b>	This method is used on water, sewage, industrial wastes, sludges, vegetation, soil and sediment and occasionally on Hi-Vol filters.
<b>Substance determined.</b>	Selenium as selenium IV.
<b>Interpretation of results.</b>	Results are reported as total Se in mg/l.
<b>Principle of method.</b>	Selenium compounds are converted to selenium IV as selenite $\text{SeO}_3^{2-}$ mildly oxidizing by acid digestion. Selenite is reduced to $\text{H}_2\text{Se}$ by sodium borohydride. This gas is then swept into a heated quartz tube where it is reduced to selenium by heat and the argon-hydrogen atmosphere in the tube. The selenium concentration is measured by atomic absorption.
<b>Time required for analysis.</b>	A single analysis requires only 3 minutes. Sample digestion, machine set-up, etc. require more time. About 200 samples per week can be analyzed by one individual.
<b>Range of application</b>	Refer to Table 1.
<b>Standard deviation.</b>	2% above 0.01 mg/l selenium based on within-run duplicates. $\pm 0.001$ below 0.01 mg/l selenium based on within-run duplicates.
<b>Accuracy.</b>	Control solutions A and B (see Table 1) are not allowed to deviate from their expected values by more than 2 standard deviations.
<b>Limit of detection.</b>	1 $\mu\text{g/l}$ .
<b>Interferences and shortcomings.</b>	Copper and high concentrations of elements which react with borohydride depress the signal. These interferences are alleviated by the addition of HCl.
<b>Minimum sample size.</b>	50 ml or 0.5 g solid sample.
<b>Preservation and sample container.</b>	Plastic or glass bottles are acceptable. Preserve each sample with 1 ml nitric acid per liter.

**Safety  
considerations.**

Strong acids are used in the digestion and analytical procedures.  
Safety glasses must be worn.

## SELENIUM

### Hydride Generation - Flameless Atomic Absorption Method

#### 1. Introduction

The sample is digested with an oxidizing acid mixture which destroys any organo-selenium complexes and converts all forms to selenite,  $\text{SeO}_3^{2-}$ . Selenite is reduced to hydrogen selenide,  $\text{H}_2\text{Se}$ , by sodium borohydride and acid. The hydride is then swept by argon carrier gas into a heated quartz tube where a mixture of air, hydrogen and argon ignites to produce an argon-hydrogen entrained air flame, which reduces the hydrogen selenide to selenium atoms. Measurement is carried out by atomic absorption spectroscopy.

#### 2. Interferences and Shortcomings

The digestion procedure requires care. The reduction of selenate to selenite is kinetically controlled and thus a rigorously consistent technique is required. The analytical procedure is somewhat more interference prone than the comparable arsenic method. Copper, nickel and high concentrations of tin, antimony and arsenic cause reduction of the signal. The copper and nickel interferences are greatly reduced by making the digestates 6N in hydrochloric acid. Heavy matrix samples such as sewage sludges and vegetation may require standard additions. Experience has shown that signal reductions due to interferences are usually less than 10%.

#### 3. Apparatus

- 3.1. Pipettes
- 3.2. Hot plate.
- 3.3. Test tubes, calibrated, 15 ml capacity.
- 3.4. Atomic absorption spectrophotometer with selenium hollow cathode lamp or electrodeless discharge lamp.
- 3.5. Gilson sampler.
- 3.6. Gilson proportioning pump.
- 3.7. Variable transformer (Variac).
- 3.8. Recorder with 1 mv span.
- 3.9. Pump tubing manifold and associated manifold glassware as shown in Figure 1.
- 3.10. Quartz tube furnace assembly as shown in Figure 2.
- 3.11. Impinger and gas separator assembly as shown in Figure 3.

#### 4. Reagents

4.1. Nitric acid ( $\text{HNO}_3$ ), concentrated, reagent grade.

4.2. Sulphuric acid ( $\text{H}_2\text{SO}_4$ ), concentrated, reagent grade.

4.3. Hydrochloric acid ( $\text{HCl}$ ), concentrated, reagent grade.

4.4. Sodium borohydride, ( $\text{NaBH}_4$ ), reagent grade.

4.5. **Hydrochloric Acid (6M)**

Add cautiously with swirling, 500 ml of concentrated  $\text{HCl}$  to 500 ml water. Cool and make up to one liter with distilled water.

4.6. **Sodium Borohydride (2% w/v)**

Dissolve 20 g sodium borohydride in about 400 ml distilled water. Add 4 pellets of sodium hydroxide and bring to 1 liter. This reagent is prepared fresh daily.

4.7. **Selenium Stock Solution 1000 mg/l**

Dissolve 1.000 g selenium in 80 ml of 9M nitric acid. Warm gently if necessary. Cool and dilute to 1 liter.

4.8. **Standard Selenium Solution (3 mg/l Se)**

In a volumetric flask, dilute 3 ml stock selenium solution to 1000 ml with distilled water.

4.9. **Selenium Calibration Standards**

Prepare calibration standards by diluting appropriate aliquots of standard selenium solution to give calibration standards with selenium concentrations as outlined in Table 1.

4.10. **Quality Control Solutions**

For soil, vegetation, sediment, sludge and Hi-Vol samples calibration control solutions are prepared by judicious blending of at least 1 month's supply of sample digestates to provide controls as QC-A in the 10% - 20% and QC-B in the 80% - 90% range of instrumental response.

For water samples, calibration control solutions are prepared by diluting 5 and 10 ml of standard selenium control solution as in 4.8, diluted to 1 liter with tap water.

For vegetation and water samples, 2 reference samples are used as quality control samples.

4.11. **Sensitivity Checks**

An undigested 20  $\mu\text{g/l}$  sensitivity check is prepared from the standard selenium solution.

#### 5. Procedure

## 5.1. Sample Preparation

5.1.1. Vegetation, soil, sediment, sewage and sludge, use the following aliquots where applicable: vegetation, soil and sediment - 0.06 g; sewage and sludge - 1 ml.

5.1.1.1. Add an appropriate aliquot of sample to a test tube calibrated at 15 ml.

5.1.1.2. Add 3 ml of 6:3:1 nitric: sulphuric: perchloric acid.

5.1.1.3. Place on an aluminum heating block and heat overnight.

5.1.1.4. Let samples cool. Add 0.5 ml distilled water and 2 ml of concentrated hydrochloric acid. Dilute to the mark with distilled water. Mix well.

5.1.2. Water and Industrial Effluent

5.1.2.1. Pipet 15 ml of sample into a 100 ml beaker. Add 3 ml of 6:3:1 nitric: sulphuric: perchloric acid.

5.1.2.2. Place beaker on a hot plate and heat until dense white fumes form.

5.1.2.3. Let samples cool. Add 0.5 ml distilled water and 2 ml concentrated hydrochloric acid.

5.1.2.4. Transfer to a 15 ml calibrated tube. Dilute to the mark with distilled water. Mix well.

5.1.3. Hi-Vol Filters

5.1.3.1. Cut 2 circles 1.8 cm in diameter from the exposed Hi-Vol filter and place in a test tube calibrated at 15 ml.

5.1.3.2. Add 3 ml of 6:3:1 nitric: sulphuric: perchloric acid.

5.1.3.3. Place on an aluminum heating block and heat overnight.

5.1.3.4. Let samples cool. Add 0.5 ml distilled water and 2 ml hydrochloric acid. Dilute to the mark with distilled water. Mix well.

## 5.2. Selenium Determination

REFER TO MANUFACTURER'S MANUAL FOR OPERATION, SETTINGS, CLEANING AND SET-UP PROCEDURES FOR THE ATOMIC ABSORPTION SPECTROPHOTOMETER.

5.2.1. Prepare and analyze digested standards with each run. Analyze every 18th sample in triplicate with 1 aliquot spiked. Details of run protocol are described in Table 1.

- 5.2.2. Turn on and adjust the variable rheostat so that the quartz tube emits a dull red glow corresponding to a temperature of 650 °C. The cell has been described elsewhere (see the Determination of Arsenic).
- 5.2.3. Allow system to equilibrate for at least 1 hour.
- 5.2.4. Analyze reference standard. If the response is within 10% of its long-term mean, analyze standards, controls and samples.
- 5.2.5. Run standard curve before and after sample run. Also run a standard at regular intervals throughout the run to monitor sensitivity changes.
- 5.2.6. Measure and record peak heights.

## 6. Calculation and Reporting

Results are calculated with the aid of a calculator program which constructs a standard curve using a least squares fit, constrained to pass through the blank value. These concentrations are multiplied by the appropriate dilution factor and reported.

Results are reported in mg/l for waters, sewages and sludges. One significant figure is reported in the range 0.001 - 0.009 mg/l and 2 significant figures are reported above 0.10 mg/l.

For soils, sediments and vegetation samples, results are reported in µg/g and to 3 significant figures above 99.9 µg/g selenium concentrations.

For Hi-Vol filters results, reported in µg/m<sup>3</sup>, are calculated as follows:

$$\text{Se (µg/m}^3\text{)} = \frac{C(\text{DF}) \times 1.181}{V}$$

Where:

C = solution concentration in µg/l

DF = dilution factor

V = volume of air sampled in m<sup>3</sup>

$$1.181 = \frac{e}{f} \times d = \frac{406.25}{5.16} \times 0.015$$

Where: e = total exposed filter area

f = filter aliquot taken

d = volume of digestate/1000

Results are reported to 3 significant figures.



## 7. Precision and Accuracy

Based on within-run duplicate samples the relative standard deviations is 4% for selenium concentrations above 0.01 mg/l and 0.001 mg/l for selenium concentrations below 0.01 mg/l.

Control is maintained by 2 standards (QC-A and QC-B) (see Table 1) at approximately 20% and 80% of range such that A and B do not vary by more than 2 standard deviations from their respective means.

## 8. Bibliography

- 8.1. American Public Health Association, American Waterworks Association and Water Pollution Control Federation (1975). Standard Methods for the Examination of Water and Wastewater. 14th edition. APHA, Washington, D.C.
- 8.2. Vijan, P.N. and Wood G.R. (1976). An automated submicrogram determination of selenium in vegetation by quartz-tube furnace atomic absorption spectrophotometry. *Talanta* **23**: 89.

TABLE 1 Run formats and Quality Control  $\mu\text{g/l}$

Matrix	Sensitivity Check	Sensitivity Absorbance Unit	Maximum Run Size		Digested Standards $\mu\text{g/l}$	Quality Controls	Other
Water	Undigested 20 $\mu\text{g/l}$ standard	0.20	150	Prepared each run Run before and after samples	0, 10, 20, 30 40	A, B mixed metal aqueous standards	Every 18th sample in triplicate with one aliquot spiked
Sludge	Undigested 20 $\mu\text{g/l}$ standard	0.20	80	"	0, 10, 20 30, 40	"	"
Hi-Vol	Undigested 20 $\mu\text{g/l}$ standard	"	80	"	0, 10, 20, 30 40	A, B composite Hi-Vol digestate	"
Vegetation	Undigested 20 $\mu\text{g/l}$ standard	"	80	"	0, 10, 20, 30 40	A, B composite vegetation or soil digestate	"
Soil and Sediment	Undigested 20 $\mu\text{g/l}$ standard	"	80	Previously digested run before & after samples	0, 10, 20, 30, 40	2 reference standards	"

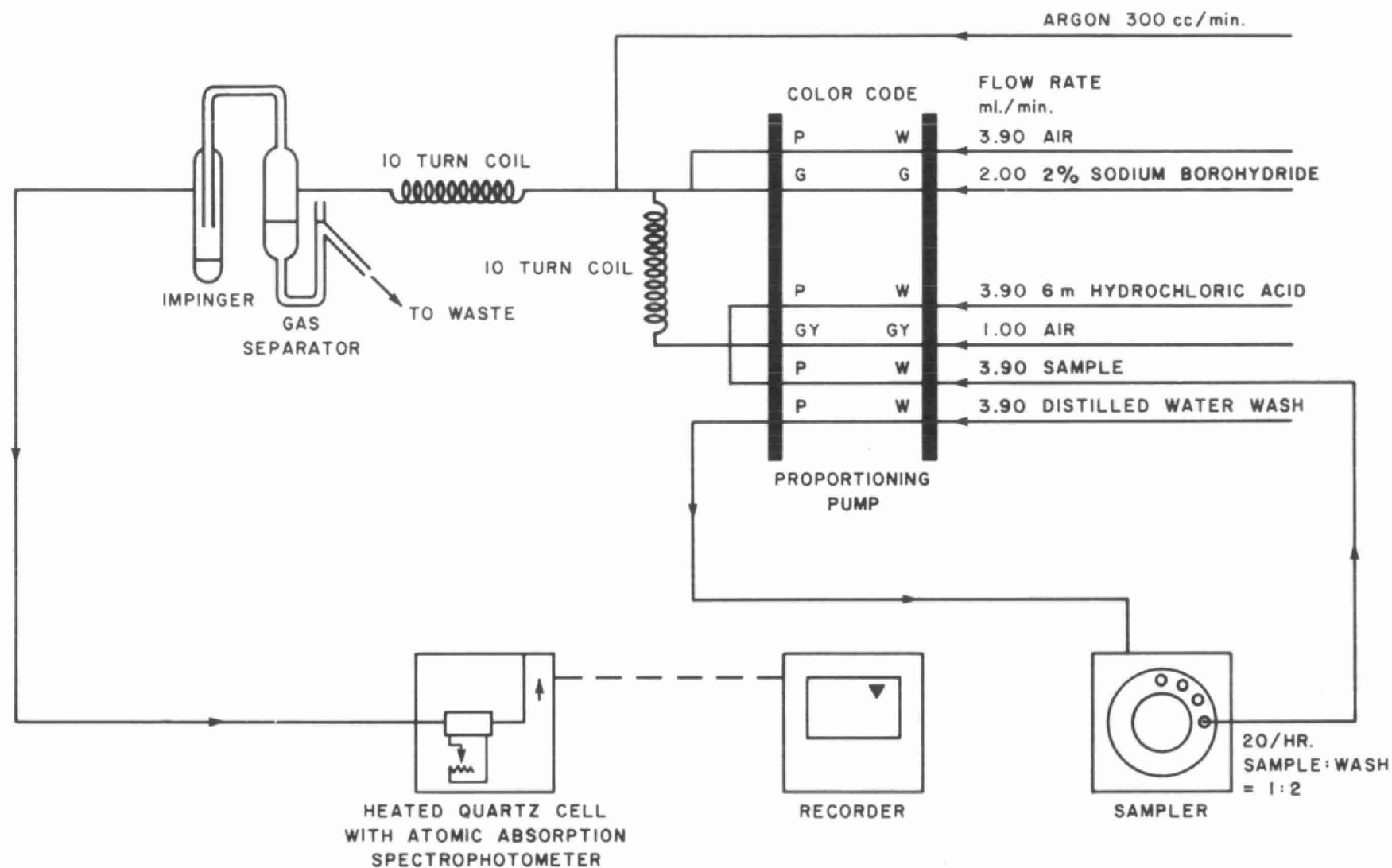


FIGURE 1 — FLAMELESS ATOMIC ABSORPTION SYSTEM FOR THE DETERMINATION OF SELENIUM

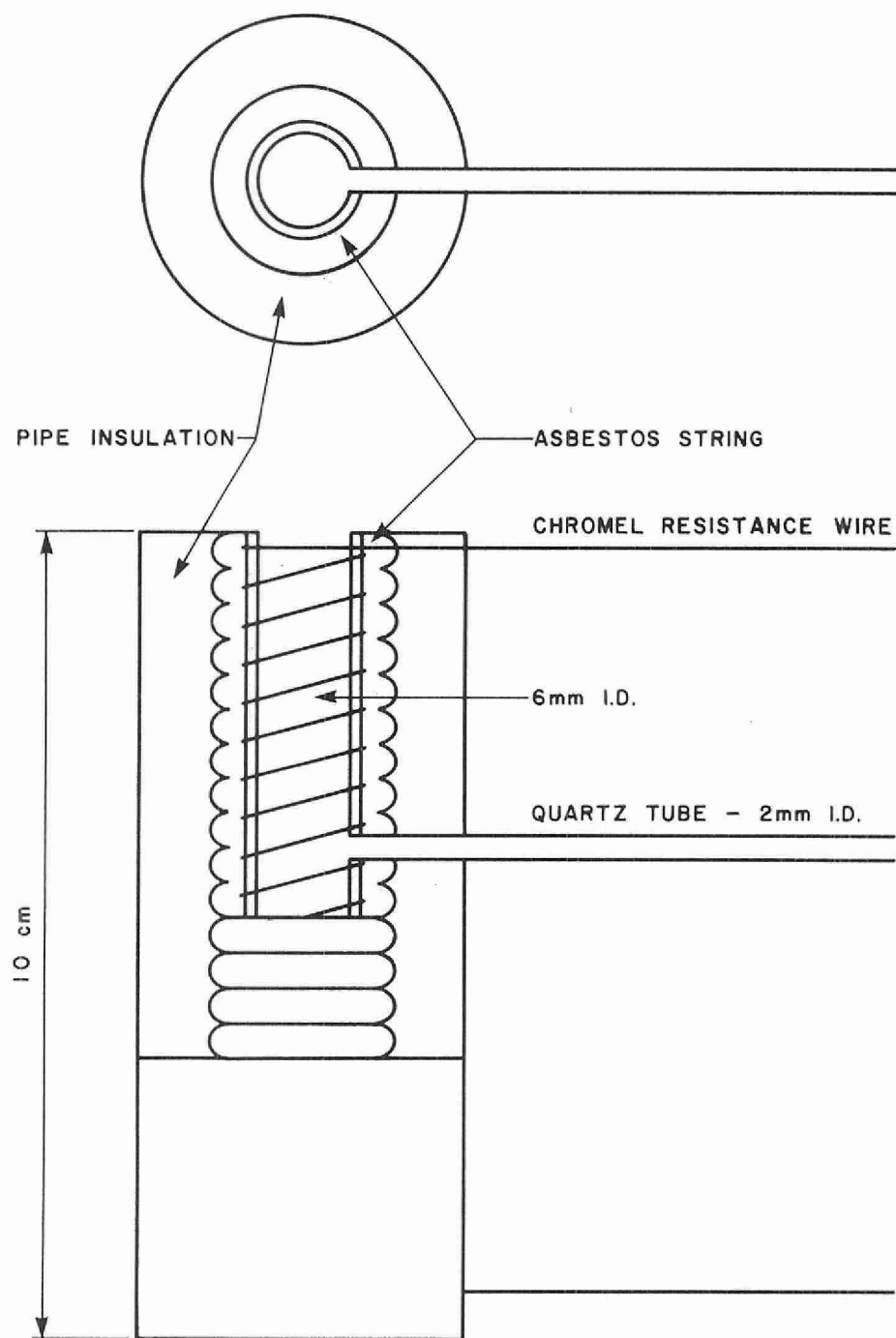


FIGURE 2 - QUARTZ TUBE FURNACE ASSEMBLY

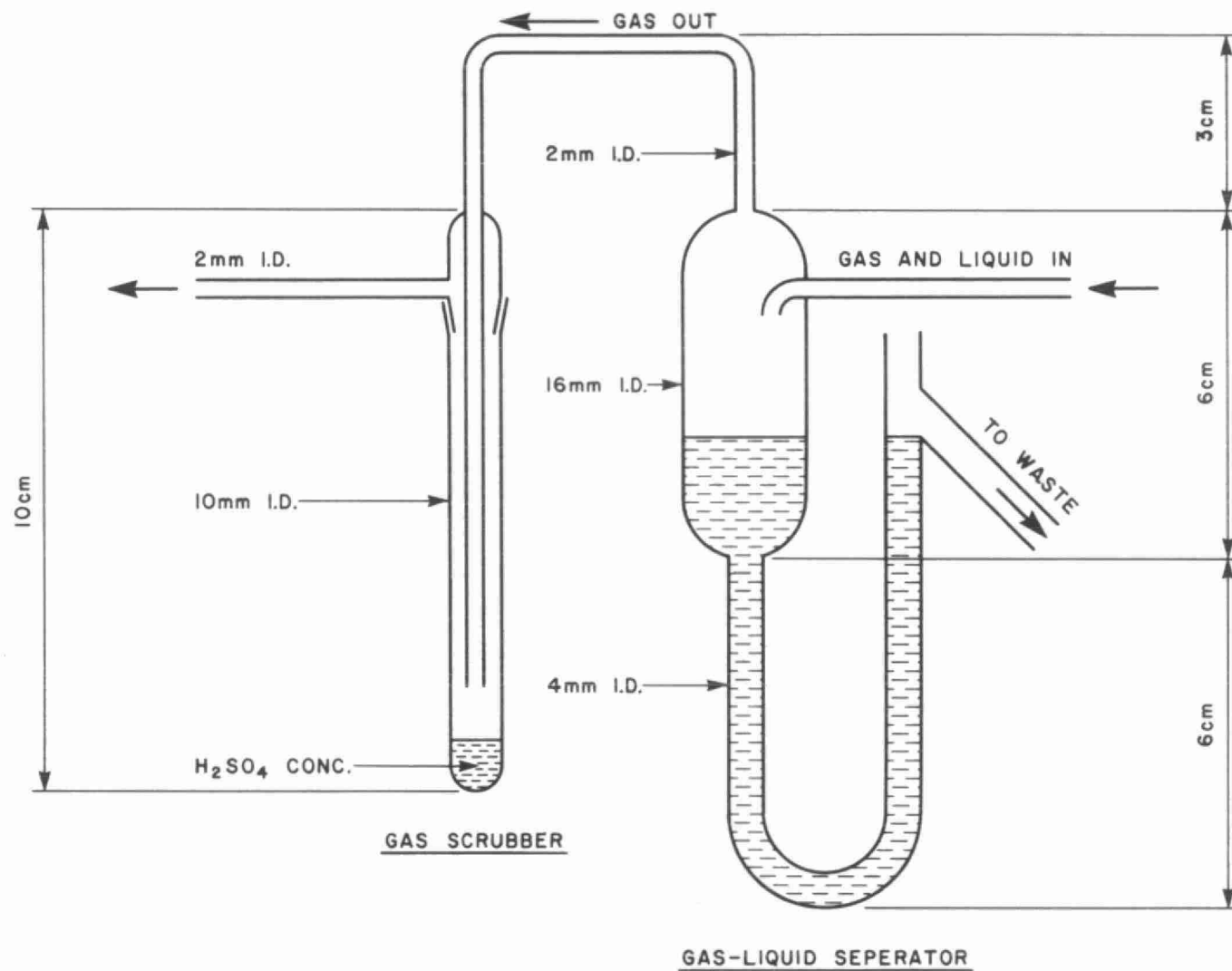


FIGURE 3 - IMPINGER AND GAS SEPARATOR ASSEMBLY

## THE DETERMINATION OF MOLYBDATE REACTIVE SILICATES

Silicon ranks second only to oxygen in abundance in the earth's crust and is a major constituent of silica and silicates, which exist as monomers or polymeric chains and cyclic anions (8.1). In natural waters, soluble and colloidal forms of all the polymorphs of silica may be present.

The presence of dissolved silicates along with other nutrients is essential to the growth of diatoms which incorporate silica into their skeletal structure (8.4). Silica is widely used in industry for the manufacture of glass, abrasives and enamels. Silicates have been used in domestic water treatment as coagulants and corrosion inhibitors although in high pressure water systems, failure to remove dissolved silica compounds may result in a detrimental accumulation of silica deposits (8.2). No adverse physiological effects are known at concentrations generally found in natural or treated waters.

### Sample Handling and Preservation

Plastic bottles should be used for sample collection. No preservatives should be added. However, the analysis should be completed as soon as possible since biological activity can reduce dissolved silicate concentrations in enriched natural samples.

### Selection of Method

The method used is based upon the formation of the molybdenum heteropoly blue complex and measures only dissolved reactive silicate anions. Silica, in the soluble colloidal form or the insoluble polymerized form, does not react and, therefore, is not detected by this method. The extent to which the various forms of silica may be hydrolyzed to a molybdate reactive form under the test conditions is presently unknown. Similarly, it is not known to what extent dissolved poly-silicates and silica are present in natural waters. The detection criterion of this method is significantly better than measurement by atomic absorption.

Dissolved non-reactive and total silica determinations are not currently performed, however, if these are required an APHA method is recommended (8.1). A method for the determination of silicon, in vegetation by XRF, is given in the Determination of Trace Metals by Atomic Spectroscopy.

## MOLYBDATE REACTIVE SILICATES

### Automated Molybdate Colorimetric Method A

#### SUMMARY

<b>Matrix.</b>	This method is currently used on surface and domestic waters.
<b>Substance determined.</b>	Molybdate reactive silicates. Any forms of silicon which can form reactive species under the conditions of the test are also measured.
<b>Interpretation of results.</b>	Results are reported as mg/l silicon (Si).
<b>Principle of method.</b>	Ammonium molybdate at pH 1.2 reacts with silicates to produce a yellow molybdosilicic acid complex. Since phosphates also react to produce a yellow phosphomolybdate complex under the same test conditions, oxalic acid is added to destroy the phosphorus chromophore. A reducing agent, ascorbic acid, is used to convert the yellow molybdosilicic acid to the heteropoly blue complex. An AutoAnalyzer system is used to measure the absorbance of the colored solution at 660 nm. The absorbance is proportional to the reactive silicate concentration in the original sample.
<b>Time required for analysis.</b>	Approximately 200 tests can be performed per day.
<b>Range of application.</b>	0.09 - 5.00 mg/l silicon. The upper limit may be extended by sample dilution.
<b>Standard deviation.</b>	Based on within-run duplicate samples in the 0.09 - 5.00 mg/l range, standard deviations are 0.053 for 0 - 20% of the range; 0.054 for 20 - 50% of the range and 0.066 for 50 - 100% of the range.
<b>Accuracy.</b>	Recoveries of two Quality Control Standards were 103.6% and 100.4%. The relative standard deviations for these standards were 1.43% and 1.98% respectively.
<b>Detection criteria.</b>	0.087 mg/l.

**Interferences and shortcomings.**

The use of glassware in the analytical system may contribute reactive silicates. However, these effects are minimized by identical treatment of standards and samples. New glass tubes cause a positive blank reading and therefore tubes should be thoroughly washed and soaked in distilled water prior to use. Recent evidence suggests that reactive silicates may become less reactive with time, and therefore prompt shipment and analysis of samples is recommended.

Turbidity interferences can be overcome by sample filtration prior to analysis. Phosphate interference is removed by the use of oxalic acid. Color interference can be overcome by performing the test manually using a sample blank for correction.

**Minimum volume of sample.**

60 ml.

**Preservation and sample container.**

No preservative should be added to samples requiring silicate analysis. Plastic bottles should be used whenever possible to avoid silicate leaching from glass containers. Prompt shipment and analysis is desirable to forestall biological conversion of silicates in enriched natural samples.

**Safety considerations.**

Standard laboratory safety practices should be followed.



## MOLYBDATE REACTIVE SILICATES

### Automated Molybdate Colorimetric Method A

#### 1. Introduction

Silicates present in the sample are reacted with ammonium molybdate at pH 1.2 to produce a yellow molybdosilicic acid complex. The yellow complex is reduced to produce the blue chromophore which has a greater stability and is more intense in color. The absorbance of the solution at 660 nm is directly proportional to the concentration of silicates which will react with molybdic acid under the test conditions.

#### 2. Interferences and Shortcomings

Glass vessels may contribute reactive silicates and therefore reagents should be prepared and stored in plastic containers.

Phosphate interference may be eliminated by the addition of oxalic acid to the sample stream after the addition of molybdic acid and prior to the addition of the reducing agent.

Turbidity interferences are generally overcome by sample filtration prior to analysis. Samples for which this is not effective and samples with color interference are analyzed manually using a sample blank for correction (a sample aliquot plus all reagents except ascorbic acid).

This method measures only dissolved reactive silicate ions. Silica, in the soluble colloidal form or the insoluble polymerized form, does not react with molybdic acid and is, therefore, not detected. The exact extent to which the various forms of silica may be hydrolyzed to a molybdate reactive form under the test conditions is unknown at the present time. Similarly, it is not generally known to what extent dissolved poly-silicates and silica are present in natural waters although at least one form is known to exist.

#### 3. Apparatus

- 3.1. Automated analysis system, Technicon AutoAnalyzer II or equivalent, consisting of the following modules:
  - 3.1.1. Sampler
  - 3.1.2. Proportioning pump

- 3.1.3. Colorimeter equipped with 660 nm filters and a 5 cm flow cell
- 3.1.4. Voltage regulator
- 3.1.5. Chart recorder

3.2. Pump tubing and assorted manifold glassware as in Figure 1.

3.3. Culture tubes, 19 x 150 mm.

3.4. Culture tube racks, 40 tube capacity.

3.5. Dilution tubes, 50 ml capacity.

#### 4. Reagents

4.1. Sodium metasilicate nanohydrate, ( $\text{Na}_2\text{SiO}_3 \cdot 9\text{H}_2\text{O}$ ), reagent grade.

4.2. Sulphuric acid ( $\text{H}_2\text{SO}_4$ ), concentrated, reagent grade.

4.3. Ammonium molybdate tetrahydrate ( $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}$ ), reagent grade crystals.

4.4. Oxalic acid dihydrate ( $\text{H}_2\text{C}_2\text{O}_4 \cdot 2\text{H}_2\text{O}$ ), reagent grade crystals.

4.5. Ascorbic Acid ( $\text{C}_6\text{H}_8\text{O}_6$ ), reagent grade powder.

##### 4.6. Ammonium Molybdate Solution

Dissolve 7.0 g ammonium molybdate in about 900 ml of distilled, deionized water. When dissolved, add 4.0 ml concentrated sulphuric acid and dilute to 1 liter. A blue solution indicates contamination.

##### 4.7. Oxalic Acid Solution

Dissolve 50 g oxalic acid in distilled, deionized water and dilute to 1 liter.

##### 4.8. Ascorbic Acid Solution

Dissolved 25 g ascorbic acid in distilled, deionized water and dilute to 1 liter in a volumetric flask. This solution is stable up to 1 week under refrigeration. A yellow solution indicates deterioration and a blue solution indicates contamination.

##### 4.9. Silicate Calibration Stock Solution (100 mg/l Si)

Dissolve 1.0118 g sodium metasilicate nanohydrate in distilled, deionized water and dilute to 1 liter in a volumetric flask. Store this solution in a tightly stoppered plastic bottle.

##### 4.10. Silicate Calibration Standards

In volumetric flasks, dilute 40 ml and 8.0 ml aliquots of silicate calibration stock solution to 1 liter each using distilled, deionized water. This gives calibration standards with silicon concentrations of 4.0 and 0.80 mg/l respectively.

#### 4.11. Quality Control Stock Solution (200 mg/l Si)

Dissolve 2.024 g sodium metasilicate nanohydrate in distilled, deionized water and dilute to 1 liter in a volumetric flask. (Use a separate batch of sodium metasilicate nanohydrate from the batch used for preparation of the silicate calibration stock solution.)

#### 4.12. Quality Control Working Standards

QC-A: In a volumetric flask dilute 50 ml quality control stock solution to 4 liters with distilled, deionized water. This solution has a silicon concentration of 2.5 mg/l

QC-B: In a volumetric flask dilute 10 ml quality control stock solution to 4 liters with distilled, deionized water. This solution has a silicon concentration of 0.5 mg/l.

#### 4.13. Daily Sensitivity Checks

In volumetric flasks prepare solutions that will provide 80% (high) and 16% (low) of full scale response by diluting 40 ml and 8 ml silicate calibration stock solution to 1 liter with distilled, deionized water. These solutions have silicon concentrations of 4.0 mg/l and 0.80 mg/l.

### 5. Procedure

REFER TO MANUFACTURER'S MANUAL FOR OPERATION, CLEANING, SET-UP AND CHECKING PROCEDURE FOR AUTOANALYZER.

5.1. Set-up and check AutoAnalyzer system. Collect and group samples by classification.

5.2. Rinse culture tube 3 times with well mixed sample and discard rinsings. Add a fourth aliquot for analysis.

5.3. Include all of the following in each run of samples:

Calibration standards (STDS); long-term blank (LTBl); distilled water blank (Bl); quality control samples (QC-A, QC-B); duplicates (DUP); sensitivity monitoring standards (HIGH and LOW); samples in groups of 10 or less (10).

5.4. Load samples in the following sequence:

HIGH; Bl; HIGH; HIGH; Bl; STDS; Bl; QC-A, QC-B; LTBl; Bl; n(10; Bl); (10; LOW; HIGH; Bl).

Where: n = number of repetitive units of samples.

**NOTE:** Randomly select at least 4 samples and analyze in duplicate in a non-sequential order.

5.5. Commence run and ensure that blanks and quality control checks are within pre-set control limits prior to analyzing samples. If the quality control checks are not within specified limits, stop run and make necessary adjustments and/or corrections. Rerun the calibration sequence and quality control checks to confirm that the system is under control.

5.6. Record measured values of QC-A, QC-B, long-term blank, STD Cal setting as well as duplicates. Also note values of highs and lows to determine whether a within-run sensitivity correction need be applied.

5.7. Read and record sample peaks.

## 6. Calculation and Reporting

If the sample was diluted multiply reading by the dilution factor:

$$\text{dilution factor} = \frac{\text{diluted volume}}{\text{aliquot volume}}$$

Report results in mg/l Si to 2 significant figures.

## 7. Precision and Accuracy

Based on within-run duplicate samples, standard deviations for the 0.09 - 5.00 mg/l concentration range are: 0.053 for 0 - 20% of range; 0.054 for 20 - 50% of range and 0.066 for 50 - 100% of range.

Recoveries of two Quality Control Standards were 103.6% and 100.4%. The relative standard deviations for these standards were 1.43% and 1.98% respectively.

## 8. Bibliography

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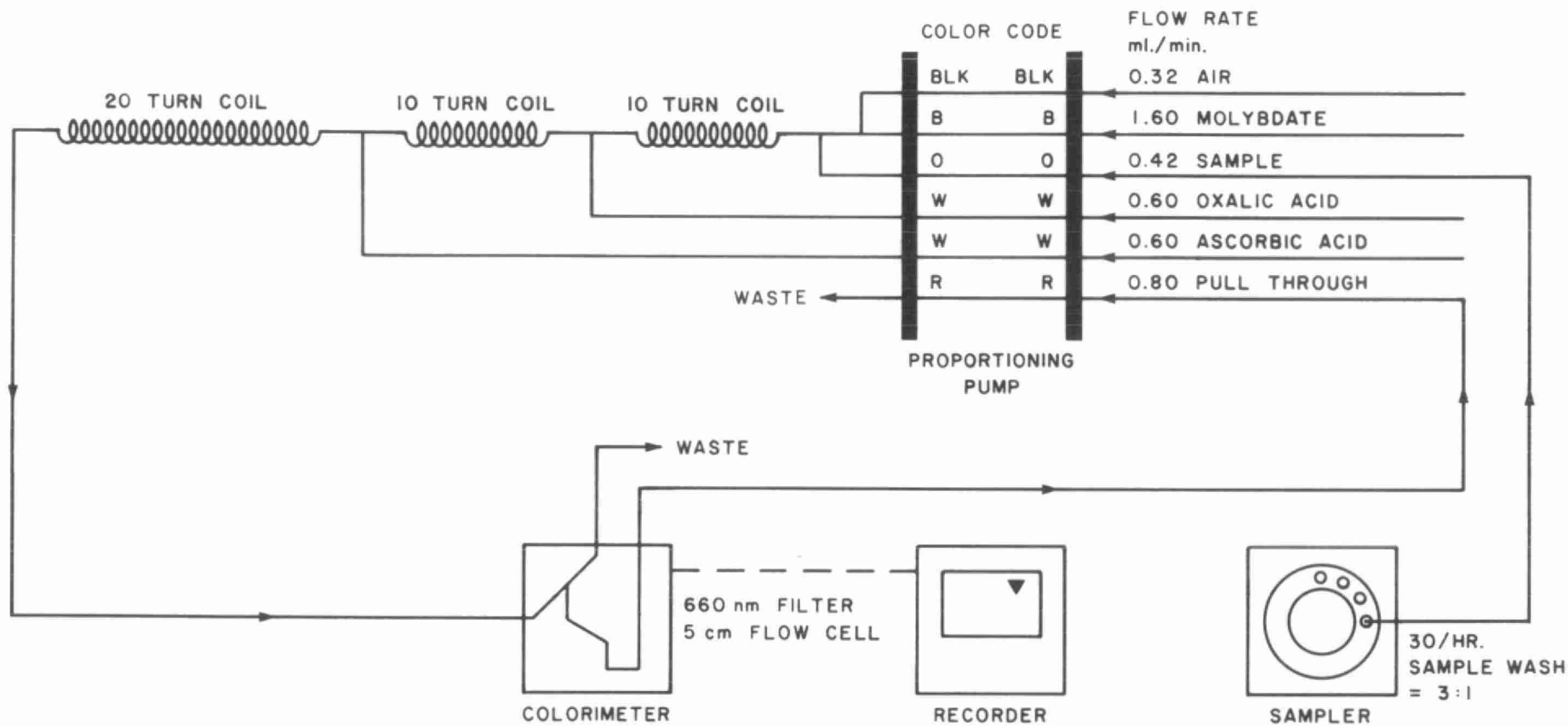


FIGURE 1 — AUTOANALYZER AAII SYSTEM FOR MOLYBDATE REACTIVE SILICATE DETERMINATION

## THE DETERMINATION OF SODIUM

Sodium is the sixth most abundant element on earth, comprising about 2.6% of the earth's crust, and is found in most natural waters. Weathering of salt deposits is the principal source of sodium in the aquatic environment and thus a correlation between sodium and chloride in natural waters is to be expected. Concentrations in waters may vary from less than one milligram per liter, in mid-continent precipitation, to several thousand milligrams per liter in some briny ground waters. In several areas in Ontario, deep wells encounter brine in the lower rock strata. With the exception of coastal regions, saline soils usually occur in arid and semi-arid areas with inadequate drainage and little leaching. Sodium in excess can be toxic to plants and may cause leaf tip burn.

Since sodium salts are used extensively in the manufacture of various chemical products, sodium is abundant in many industrial effluents. Commercial water softening devices which replace calcium and magnesium with sodium ions are widely employed. The common practice of employing salt for control of road-ice may contribute substantial quantities of this element to surface and ground waters receiving drainage from highways. Plants and soils on either side of the highway may be adversely affected by such drainage or by vehicular spray. Soils having a high sodium content usually exhibit low infiltration and permeability rates and are consequently unsuitable for farming, and plants may receive toxic levels of sodium.

Sodium is generally considered non-toxic to humans and therefore drinking water standards have not been established; however, persons with high blood pressure are usually advised to consume water that has less than 20 mg/l. Hard waters which have been softened by a domestic water softener will contain concentrations in excess of this level.

### Sample Handling and Preservation

#### Water, Sewages and Industrial Wastes

Plastic containers are preferred for surface waters and are required for precipitation samples since soft glass and to a lesser extent pyrex containers release sodium ions to the sample (usually less than 0.5 mg/l) particularly under conditions of high pH. When the expected sodium concentration is sufficiently large to render this container effect negligible, glass bottles may be used.

The usual precaution of rinsing the container several times with sample should be observed; no preservative is recommended since sodium is unusually inert, and remains in solution.

### Selection of Method

Method A is an automated atomic absorption technique which employs a high (10. - 100 mg/l) and a low (0.1 - 10 mg/l) range suitable for direct analysis of most surface waters, a low range of 0.01 - 1.00 mg/l for precipitation samples and 0 - 50 and 50 - 200 mg/l sodium ranges for drinking waters. A manual atomic absorption method is used on industrial wastes and is described in the Determination of Trace Metals by Atomic Spectroscopy.

## SODIUM

### Automated Atomic Absorption Method A

#### SUMMARY

<b>Matrix.</b>	This method is used on surface waters, precipitation and drinking water sewage and industrial waste samples.
<b>Substance determined.</b>	Sodium ion, Na.
<b>Interpretation of results.</b>	Results are reported as mg/l Na.
<b>Principle of method.</b>	An automated atomic absorption method is used to measure the concentration of sodium ions. The sample is diluted with an ionization suppressant, KCl, prior to aspiration into an air-acetylene flame.
<b>Time required for analysis.</b>	A single determination requires 15 minutes; however, by suitable batch arrangement, 150 samples per day may be analyzed.
<b>Range of application.</b>	Surface waters: a) 0.1 - 10 mg/l b) 10 - 100 mg/l Drinking water: 0 - 50 mg/l 50 - 200 mg/l Precipitation: 0.01 - 1.00 mg/l
<b>Standard deviation.</b>	Relative standard deviations are 1.2% for precipitation samples; 1.2% for surface waters in the 0.1 - 10.0 mg/l; 1.5% for surface waters in the 1 - 100 mg/l range and 0.61% for drinking water samples in the 0.3 - 100 mg/l range.
<b>Accuracy.</b>	Calibration is controlled by 2 independently prepared long-term standards QC-A and QC-B for each range. Control is maintained such that (A + B) and (A - B) do not vary by more than 3 standard deviations from the long-term mean of (A - B). Control limits are as follows: 0.03 mg/l for precipitation samples; 0.154 mg/l for low level surface waters; 0.90 mg/l for high level surface waters and 1.2 mg/l for low range drinking water samples and 4.9 mg/l for high level drinking water samples.
<b>Detection criteria.</b>	0.024 for precipitation samples; 0.115 for low level surface waters; 0.793 for high level surface waters and 0.31 for drinking water in the 0.2 - 100 mg/l range.

**Interferences  
and shortcomings.**

Partial ionization of the sodium atoms in the flame yields correspondingly low results. Automatic addition of KCl solution to achieve a final potassium level of 500 mg/l for surface and drinking waters, and 250 mg/l for precipitation is employed to control this problem. Careful maintenance of stable aspiration and burner conditions is also important.

**Minimum volume  
of sample.**

50 ml for surface water and drinking water samples, 10 ml for precipitation samples.

**Preservation and  
sample container.**

No special preservation measures are required. Use of plastic sampling containers is advised and required for precipitation samples which have low concentrations of sodium. For the case in which expected sample concentration is sufficiently high, or when the desired analytical accuracy level will not be affected by small additions from glass (<0.5 mg/l even at high pH), glass samples containers are acceptable.

**Safety  
considerations.**

The possibility of burner flash-back or explosion is always present when using flame atomic absorption apparatus. The manufacturer's instructions for burner ignition, use, and shut-down should always be rigorously followed and the waste trap should be filled with water at all times. Similarly, standard safety procedures should be employed when working with compressed gas cylinders. The atomic absorption unit should be placed under a suitable exhaust canopy to expel heat and fumes.



## SODIUM

### Automated Atomic Absorption Method A

#### 1. Introduction

The sample under test, automatically diluted with an ionization suppressant, is aspirated as a fine mist into an air-acetylene flame. Light emitted from a hollow cathode lamp at the characteristic wavelength of sodium, is directed through the flame into a monochromator and onto a detector. Sodium atoms, heated in the flame, absorb this light and the detector measures the decreased intensity of the resulting beam. The amount of light absorbed is directly proportional to the concentration of sodium in the sample, and is recorded on a strip-chart recorder as a series of peaks. Calibration, at the low dilution levels made possible by the sensitivity of the AAS instrument, is nearly linear. A Hewlett Packard 9864A Digitizer may be employed to automatically calculate and record sample concentration, by comparison to a calibration plotted from known standards.

#### 2. Interferences and Shortcomings

Partial ionization of sodium atoms in the flame results in a decrease in absorbance, and low results. The degree of ionization is a function of temperature; hence for reproducibility, stable aspiration and flame conditions must be maintained for both standards and samples throughout an analytical run. Potassium is automatically added as an ionization suppressant.

Partial clogging of the burner nebulizer and consequent reduction in aspiration may result from processing samples containing large amounts of suspended solids; pre-filtration of samples is advisable in this case.

#### 3. Apparatus

- 3.1. Atomic absorption spectrophotometer, a Varian AA275 is used for precipitation samples, a Pye Unicam SP1900 is used for surface waters and a Varian AA5 is used for drinking water samples.
- 3.2. Proportioning pump with manifold tubing and glassware as assembled in Figures 1, 2, 3, and 4.
- 3.3. Sampler. A Technicon Large Industrial model sampler is currently used for surface waters and a Gilson sampler is used for precipitation and drinking water samples.
- 3.4. Chart recorder.
- 3.5. Sampling tubes to fit sampler used. Disposable plastic specimen tubes are used for precipitation samples.

A ROUTINE BURNER MAINTENANCE PROGRAM IS RECOMMENDED WHICH ENSURES CLEAN BURNER HEAD AND MIXING CHAMBER

CONDITIONS, CLEAN GAS AND DRAINAGE TUBES, AND A NEBULIZER FREE OF BLOCKAGE. SUCH A PROGRAM IS NOT ONLY NECESSARY FROM A SAFETY STANDPOINT, BUT WILL ALSO PROVIDE EFFICIENT, ACCURATE OPERATION OF THE AAS.

#### 4. Reagents

- 4.1. Sodium chloride (NaCl), reagent grade crystals.
- 4.2. Potassium chloride (KCl), reagent grade crystals.

##### Surface Waters

#### 4.3. Sodium Stock Solution (40,000 mg/l)

In a 500 ml volumetric flask, dissolve 50.8400 g sodium chloride (oven dried and cooled in a desiccator) in distilled, deionized water and dilute to the mark with distilled, deionized water.

**NOTE:** If potassium, calcium and magnesium are also to be determined, stock solutions for these elements are required and are described in the individual methods.

#### 4.4. Combined Intermediate Solution (low range 0.1 - 10 mg/l range)

In a 1 liter volumetric flask, dilute 5 ml sodium stock solution, 25 ml potassium stock solution, 10 ml calcium stock solution and 5 ml magnesium stock solution, to the mark with distilled, deionized water. The final concentrations are as follows: 200 mg/l sodium, 100 mg/l magnesium, 100 mg/l potassium and 400 mg/l calcium.

#### 4.5. Combined Intermediate Solution (high range 10 - 100 mg/l range)

In a 1 liter volumetric flask, dilute 50 ml sodium stock solution, and 50 ml each of potassium, calcium and magnesium stock solutions to the mark with distilled, deionized water. This gives a sodium concentration of 2000 mg/l, a potassium concentration of 200 mg/l, a calcium concentration of 2000 mg/l and a magnesium concentration of 1000 mg/l.

#### 4.6. Combined Calibration Standards (low range 0.1 - 10 mg/l range)

In a 1 liter volumetric flask, dilute the following aliquots of combined intermediate solution (reagent 4.4) to 1000 ml with distilled, deionized water to give combined standards and allow the determination of four elements.

Aliquot	Sodium	Potassium	Calcium	Magnesium
50 ml	10	5	20	5
40 ml	8	4	16	4
30 ml	6	3	12	3
20 ml	4	2	8	2
10 ml	2	1	4	1
5 ml	1	0.50	2	0.50

#### 4.7. Combined Calibration Standards (10 - 100 mg/l range)

In a 1 liter volumetric flask, dilute the following aliquots of combined intermediate solution (reagent 4.5) to 1000 ml with distilled, deionized water to give combined standards and allow the determination of four elements.

Aliquot	Sodium	Potassium	Calcium	Magnesium
50 ml	100	10	100	50
40 ml	80	8	80	40
30 ml	60	6	60	30
20 ml	40	4	40	20
10 ml	20	2	20	10
5 ml	10	1	10	5

**4.8. Quality Control Stock Solution (144 mg/l, for low range)**

In a 1 liter volumetric flask, dissolve 0.3661 g sodium chloride (oven dried and cooled in a desiccator) in distilled, deionized water and dilute to the mark. Use a different batch of sodium chloride than the one used for the preparation of reagent 4.4.

**4.9. Quality Control Working Solutions (Low Range)**

QC-A: In a 1 liter volumetric flask, dilute 50 ml quality control stock solution (reagent 4.8) to 1000 ml with distilled, deionized water to give a solution which is 72% of scale.

QC-B: In a 1 liter volumetric flask, dilute 20 ml quality control stock solution (reagent 4.8) to 1000 ml with distilled, deionized water to give a solution which is 28.8% of scale.

**NOTE:** For quality control stock solution for calcium, magnesium and potassium see individual methods.

**4.10. Quality Control Stock Solution (1440 mg/l, for high range)**

In a 1 liter volumetric flask, dissolve 3.6605 g sodium chloride (oven dried and cooled in a desiccator) with distilled, deionized water and dilute to the mark. Use a different batch of sodium chloride than the one used for the preparation of reagent 4.3.

**4.11. Quality Control Working Solutions (High Range)**

QC-A: In a 1 liter volumetric flask, dilute 50 ml quality control stock solution (reagent 4.10) to 1000 ml with distilled, deionized water to give a solution which is 72% of scale.

QC-B: In a 1 liter volumetric flask dilute 20 ml quality control stock solution (reagent 4.10) to 1000 ml with distilled, deionized water to give a solution which is 28.8% of scale.

**NOTE:** For calcium, magnesium and potassium quality control solutions see individual methods.

**4.12. Potassium Chloride Suppressant**

Dissolve 10 g potassium chloride in distilled, deionized water and dilute to 4 liters.

## Precipitation Samples

### **4.13. Sodium Stock Solution (1000 mg/l)**

In a 1000 ml volumetric flask, dissolve 2.5420 g sodium chloride (oven dried and cooled in a desiccator) in distilled, deionized water and dilute to the mark.

**NOTE:** If calcium, magnesium and potassium are also to be measured see corresponding methods for instructions pertaining to the preparation of stock solutions for these elements.

### **4.14. Combined Sodium-Potassium Intermediate Stock Solution**

In a 1 liter volumetric flask, dilute 20 ml sodium stock solution and 20 ml potassium stock solution (see the Determination of Potassium) to the mark with distilled, deionized water. This solution contains 20 mg/l sodium and 20 mg/l potassium.

**NOTE:** If calcium and magnesium are also to be measured a combined intermediate solution is prepared by diluting 10 ml magnesium and 10 ml calcium stock solution to 1000 ml with distilled, deionized water.

### **4.15. Combined Sodium-Potassium Working Standards**

Into 1 liter volumetric flasks, pipette 5.00, 10.00, 20.00, 30.00, 40.00 and 50.00 ml combined sodium-potassium intermediate solution (reagent 4.14.) and dilute each to the mark with distilled, deionized water. This gives working standards with sodium and potassium concentrations of 0.10, 0.20, 0.40, 0.60, 0.80, and 1.00 mg/l respectively.

**NOTE:** For calcium-magnesium working standards see the Determination of Calcium (or Magnesium).

### **4.16. Quality Control Stock Solution (1000 mg/l)**

Prepare a sodium quality control stock solution which is identical to reagent 4.13 but using a different batch of sodium chloride.

**NOTE:** For calcium and magnesium quality control stock solutions see appropriate methods.

### **4.17. Combined Sodium-Potassium Quality Control Intermediate Solution**

In a 1 liter volumetric flask, dilute 20 ml sodium quality control stock solution and 20 ml potassium quality control stock solution (see the Determination of Potassium) to the mark with distilled, deionized water. This solution contains 20 mg/l sodium and 20 mg/l potassium.

**NOTE:** If calcium and magnesium are also to be determined a combined calcium-magnesium quality control intermediate solution is also prepared. See the Determination of Calcium (or Magnesium).

### **4.18. Quality Control Working Solutions**

**QC-A:** In a 1 liter volumetric flask, dilute 30 ml combined sodium-potassium quality control intermediate solution to 1 liter with distilled, deionized water to give a solution with a sodium concentration of 0.60 mg/l and a potassium concentration of 0.60 mg/l.

**QC-B:** In a 1 liter volumetric flask, dilute 5 ml combined sodium-potassium quality control intermediate solution to 1 liter with distilled, deionized water to give a solution with a sodium concentration of 0.10 mg/l and a potassium concentration of 0.10 mg/l.

**NOTE:** For quality control working solutions for calcium and magnesium see the Determination of Calcium and the Determination of Magnesium.

#### 4.19. Potassium Chloride Suppressant

Dissolve 4.767 g potassium chloride in distilled, deionized water and dilute to 1 liter. The concentration of the suppressant is 2500 mg K/l.

#### Drinking Water Samples

#### 4.20. Combined Stock Solution (20,000 mg/l sodium)

Prepare a combined stock solution by dissolving, in a 1 liter volumetric flask: 50.8424 g sodium chloride (oven dried and cooled in a desiccator); 7.6273 g potassium chloride (oven dried and cooled in a desiccator); 49.9447 g calcium carbonate (oven dried, cooled in a desiccator and dissolved in 1:1 hydrochloric acid) and 8.000 g magnesium ribbon (cleaned with hydrochloric acid and dissolved in 1:1 hydrochloric acid). Dilute to 1 liter with distilled water. This solution contains 8000 mg/l magnesium, 20,000 mg/l calcium, 4,000 mg/l potassium and 20,000 mg/l sodium.

#### 4.21. Combined Intermediate Solution (low range 0 - 50 mg/l)

In a 1 liter volumetric flask, dilute 25.0 ml combined stock solution (reagent 4.20) to 1 liter with distilled water. This gives a concentration of 500 mg/l sodium, 500 mg/l calcium, 100 mg/l potassium and 200 mg/l magnesium.

#### 4.22. Combined Intermediate Solution (high range 50 - 200 mg/l)

In a 1 liter volumetric flask, dilute 100 ml combined stock solution (reagent 4.20) to 1 liter with distilled water. This gives a concentration of 2000 mg/l sodium, 2000 mg/l calcium, 400 mg/l potassium and 800 mg/l magnesium.

#### 4.23. Sodium Working Solutions

In 1 liter volumetric flasks, dilute: 20 ml of combined intermediate solution (low range, reagent 4.21) to 2 liters with distilled water to give a 10% low range standard (5 mg Na/l); 100 ml combined intermediate solution (low range, reagent 4.21) to 1 liter with distilled water to give a 100% low range standard (50 mg Na/l); and 100 ml combined intermediate solution (high range, reagent 4.22) to 1 liter with distilled water to give a 100% high range standard (200 mg Na/l). This gives 3 working standards of which the 50 mg Na/l standard is used for both the high and low range.

#### 4.24. Combined Quality Control Stock Solution

In a 250 ml volumetric flask, dissolve 1.6524 g sodium chloride (oven dried and cooled in a desiccator); 0.2478 g potassium chloride (oven dried and cooled in a desiccator) in distilled water. Add 0.2600 g magnesium ribbon (cleaned and dissolved in 1:1 v/v hydrochloric acid and 1.6232 g calcium carbonate (oven dried, cooled in a desiccator and dissolved in 1:1 v/v hydrochloric acid). Dilute to the mark with distilled water. Use a different batch of chemicals than those used to prepare reagent 4.20.

#### 4.25. Combined Quality Control Working Solutions

QC-A: In a 2 liter volumetric flask, dilute 100 ml combined quality control stock solution to 2 liters with distilled water. This solution contains: 130 mg/l sodium; 130 mg/l calcium; 52.0 mg/l magnesium and 26.0 mg/l potassium.

QC-B: In a volumetric flask, dilute 25 ml combined quality control stock solution to 2 liters with distilled water. This solution contains 32.5 mg/l sodium; 32.5 mg/l calcium; 13 mg/l magnesium and 6.5 mg/l potassium.

QC-C: In a volumetric flask, dilute 5 ml combined quality control stock solution to 2 liters with distilled water. This solution contains 6.5 mg/l sodium; 6.5 mg/l calcium, 2.6 mg/l magnesium and 1.3 mg/l potassium.

#### 4.26. Potassium Chloride Suppressant

Dissolve 7.6 g potassium chloride in distilled water and dilute to 4 liters.

### 5. Procedure

#### 5.1. Surface Waters

SODIUM ANALYSES ARE PERFORMED USING AN AIR-ACETYLENE FLAME IN AN IDENTICAL MANNER AS CALCIUM (SEE THE DETERMINATION OF CALCIUM, METHOD B) WITH THE FOLLOWING EXCEPTIONS:

5.1.1. A sodium hollow cathode lamp operating at approximately 50% of the rated lamp current is employed, hence monochromator "peaking" at the 589.0 nm wavelength for sodium is required.

5.1.2. The ionization suppressant and diluent reagent is the potassium chloride solution designed for the range in use (prepared as outlined previously).

5.1.3. The particular pump and manifold design used for sodium analysis is given in Figure 1 and 2 for the low and high range respectively.

5.1.4. Monochromator slit width should be 0.10 nm

**NOTE:** DUE TO SLIGHT DIFFERENCES THAT MAY EXIST BETWEEN THE ASPIRATION RATES OF DIFFERENT AAS UNITS, SMALL MODIFICATIONS OF THE MANIFOLD MAY BE NECESSARY TO SATISFY THE BURNER ASPIRATION REQUIREMENT.

**NOTE:** THE AAS UNIT MUST NOT BE LEFT UNATTENDED WHILE IN OPERATION.

#### 5.2. Precipitation Samples

THE PROCEDURE FOR SODIUM ANALYSIS IS IDENTICAL TO CALCIUM ANALYSIS (SEE THE DETERMINATION OF CALCIUM) WITH THE FOLLOWING EXCEPTIONS:

5.2.1. Set the following controls to the values provided:

lamp current = 5 mA

wavelength = 589.0 nm

burner height = optimum level for the element

For all other details of the AAS operation, the reader is referred to the Determination of Calcium.

5.2.2. The manifold design is given in Figure 3.

### 5.3. Drinking Water Samples

SODIUM ANALYSES ARE PERFORMED USING AN AIR-ACETYLENE FLAME. THE FOLLOWING IS A GENERAL GUIDE FOR INSTRUMENT SET UP AND RUN SEQUENCE. FOR MORE DETAILED INFORMATION ON THE INSTRUMENT, THE OPERATOR IS REFERRED TO THE MANUFACTURER'S MANUAL.

- 5.3.1. Turn on all modules and set lamp current at 3 mA for calcium, sodium and potassium, 2 mA for magnesium.
- 5.3.2. Turn on fuel at cylinder and air at tap. Press AIR-ACETYLENE button. Press TEST FLOW button and adjust fuel-oxidant flow to 4 and 6 respectively. Press IGNITION BUTTON to light flame. Aspirate distilled water.
- 5.3.3. Select correct sample line and pump distilled water through all lines (speed 550).
- 5.3.4. Set mode to % T.
- 5.3.5. Adjust wavelength using the wavelength scan and set to 589.0 nm
- 5.3.6. Set slit width to 0.15 nm.
- 5.3.7. Align lamp using knurled screw at the back of the turret. Turn until a maximum % T is attained.
- 5.3.8. Adjust wavelength using wavelength setting until a maximum % T is reached (589.0 nm).
- 5.3.9. Pump appropriate suppressant and attach to nebulizer.
- 5.3.10. Switch to absorbance mode. Set zero on absorbance scale using fine gain.
- 5.3.11. Turn on chart recorder (high range 15 mv; low range 2 mv) and set zero using zero adjust knobs.
- 5.3.12. Sample 2 HIGH 100% standards (200 mg/l Na; 200 mg/l Ca; 80 mg/l Mg and 50 mg/l K) and adjust to 100 on scale by rotating burner (and scale expand, if necessary). Adjust peak height on chart to required height. Run a low range 100% standard (50 mg/l Na; 50 mg/l Ca; 20 mg/l Mg and 10 mg/l K) and adjust peak height on chart using chart attenuation knob.
- 5.3.13. Run QC-A; QC-B; QC-C; Blank.
- 5.3.14. Run (10 samples; blank; LOW 10%; LOW 100%; HIGH 100%; blank, 10 samples, blank, 10 samples; blank Low 10%, Low 100%, etc).



5.3.15. Run at least 3 sets of duplicates per day.

5.3.16. The manifold design is given in Figure 4.

## 6. Calculation and Reporting

Sample absorbance is compared to the absorbance of known standards and sodium concentration is read from a calibration curve. Results are multiplied by the dilution factor, if necessary.

Results are reported as follows:

### Surface Waters

Range of result	Report
$< 0.10$ mg/l	$< 0.10$
0.10 - 9.9 mg/l	to 2 significant figures
$> 10.0$ mg/l	to 3 significant figures

### Precipitation

Range of results	Report
$< 0.01$ mg/l	$< 0.01$
0.01 - 1.00 mg/l	to nearest 0.01

### Drinking Water

Range of result	Report
$< 1$ mg/l	$< 1$
1 - 99 mg/l	to 2 significant figures
100 - 200 mg/l	to 3 significant figures

## 7. Precision and Accuracy

Standard deviations based on within-run duplicate samples are as follows:

Sample Type	Range (mg/l)	$S_{ld}$	$S_{md}$	$S_{hd}$
Surface water	0.1 - 10.0	0.070	0.076	0.090
	1 - 100	0.482	0.988	1.133
Precipitation	0.02 - 1.00	0.0143	0.027	0.030
Drinking water	0.3 - 100	0.19	0.32	0.46

Where:

$S_{ld}$  = standard deviation for 0 - 20% of range

$S_{md}$  = standard deviation for 20 - 50% of range

$S_{hd}$  = standard deviation for 50 - 100% of range

Calibration is controlled by 2 independently prepared quality control standards (QC-A and QC-B) for each range. Control is maintained in such a way that (A + B) and (A - B) do not vary by more than 3 standard deviations from the long-term mean of (A - B). These control limits are 0.90 mg/l for high range (1 - 100 mg/l) surface



waters; 0.154 mg/l for low range (0.1 - 10.0 mg/l) surface waters; 0.03 mg/l for precipitation samples; 1.2 mg/l for low range (0 - 50 mg/l) drinking water and 4.9 mg/l for high range (0 - 200 mg/l) drinking water.

## **8. Bibliography**

- 8.1 American Public Health Association, American Water Works Association and Water Pollution Control Federation (1971). Standard Methods for the Examination of Water and Wastewater. 13th edition, APHA, Washington, D.C., 316.
- 8.2 Lindow, O. (1979). Determination of trace levels of calcium, magnesium, sodium and potassium by atomic absorption spectrophotometry - precipitation samples. Ministry of the Environment, Laboratory Services Branch, Rexdale, Ontario.
- 8.3 United States Environmental Protection Agency (1974). Methods of Chemical Analysis of Water and Wastes. U.S. EPA, Washington, D.C. 147.

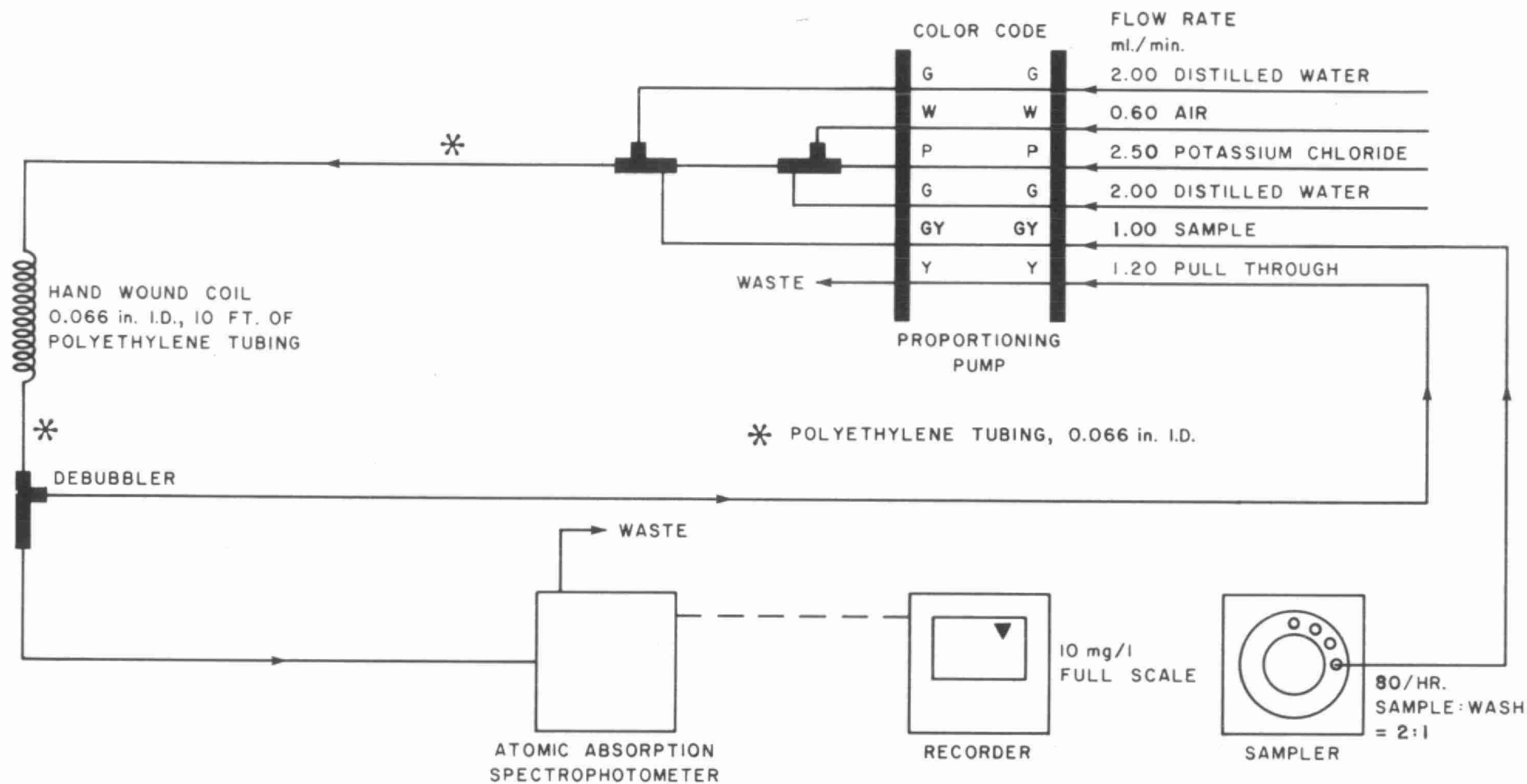


FIGURE 1 — AUTOMATED ATOMIC ABSORPTION MANIFOLD FOR LOW LEVEL SODIUM DETERMINATIONS IN SURFACE WATERS

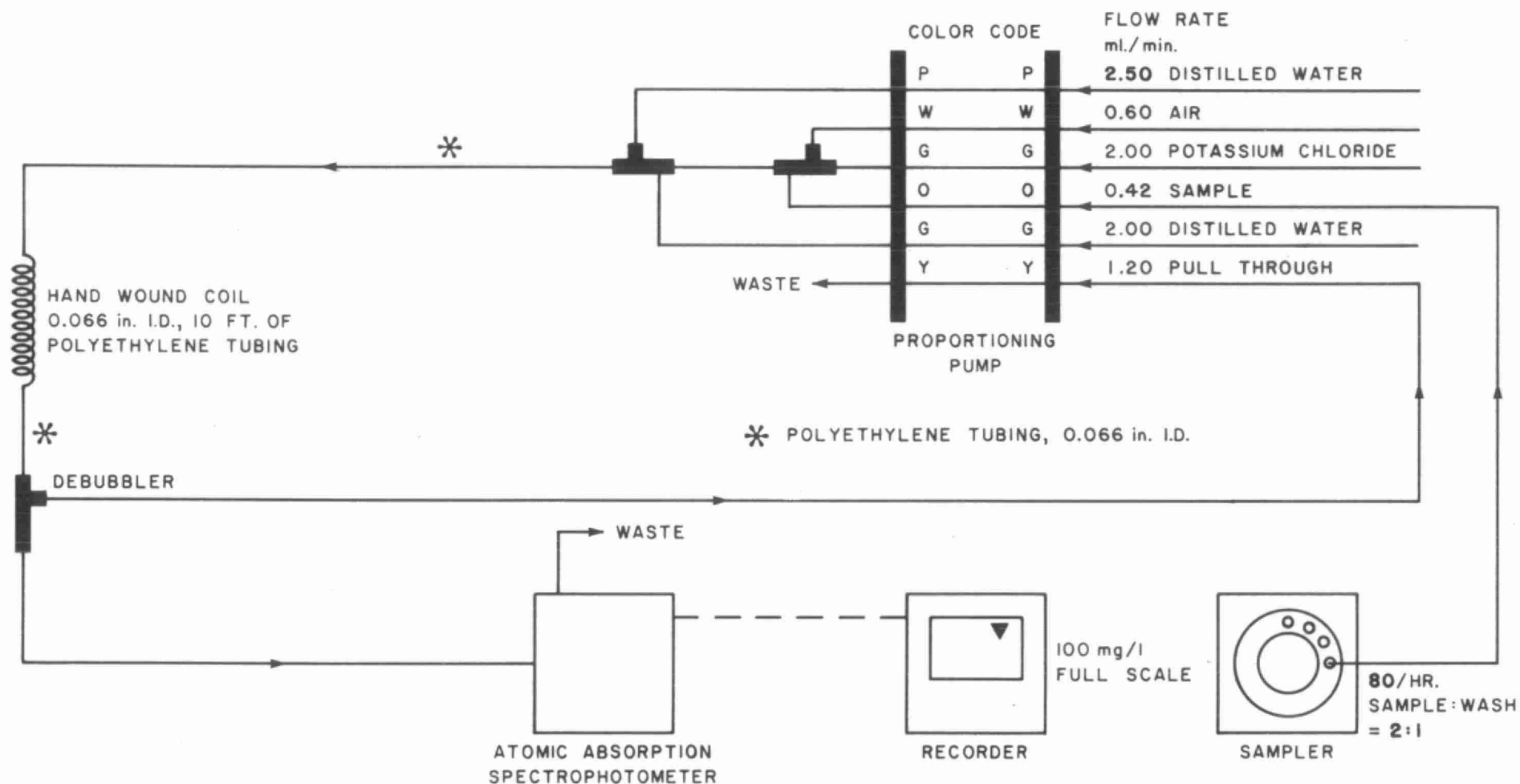


FIGURE 2 — AUTOMATED ATOMIC ABSORPTION MANIFOLD FOR HIGH LEVEL SODIUM DETERMINATIONS IN SURFACE WATERS

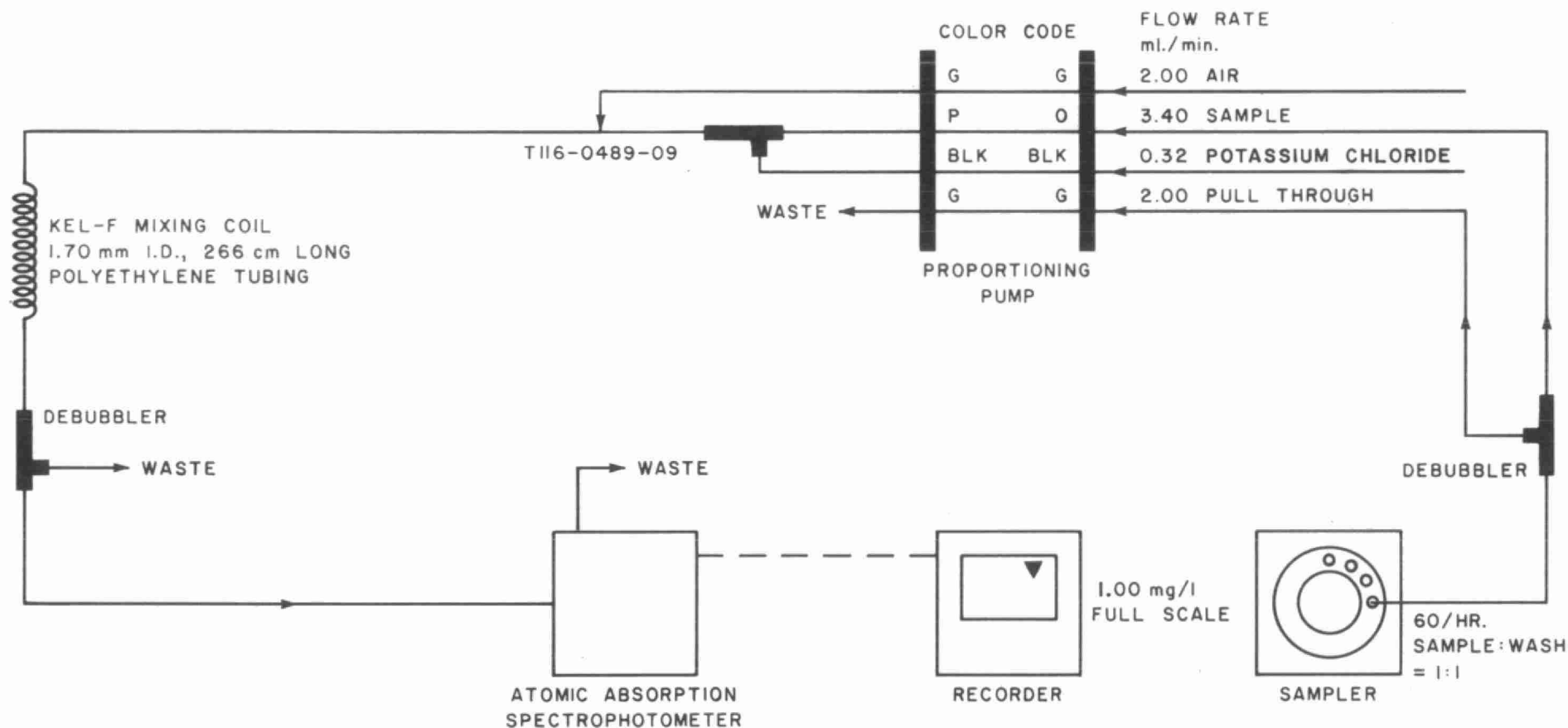


FIGURE 3 — AUTOMATED ATOMIC ABSORPTION MANIFOLD FOR SODIUM DETERMINATIONS ON PRECIPITATION SAMPLES

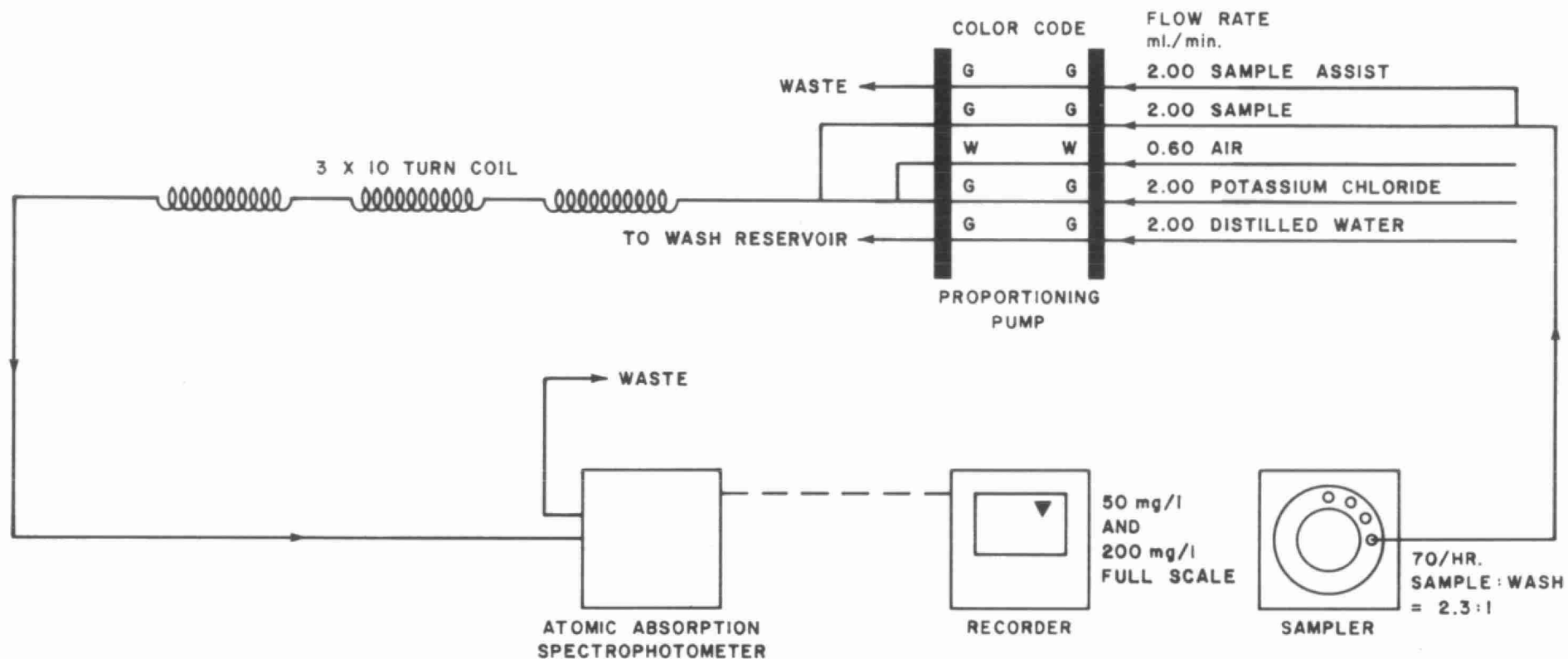


FIGURE 4 — ATOMIC ABSORPTION MANIFOLD FOR SODIUM DETERMINATION IN DRINKING WATERS.

## THE DETERMINATION OF SOLIDS-DISSOLVED

The term dissolved solids refers to the quantity of material remaining as a residue after the water is removed from a filtered portion of sample by evaporation. The gravimetric test does not determine specific chemical substances, but detects only a general class of substances which respond similarly under the same test conditions. By assuming the dissolved solids content arises solely from ionic species, this parameter can be estimated by calculating the contribution of ten common ions or from the conductivity provided the latter is less than 450  $\mu\text{S}/\text{cm}$ .

Man's activities tend to steadily increase the dissolved solids content of our water resources both by the production of wastes and by chemical treatments designed to remove harmful substances. Dissolved solids may render a water unsuitable for drinking, agricultural use or industrial activity. The permissible criteria for a public surface water source or for irrigation is 500 mg/l; the source limit is 100 mg/l for the textile industry, 500 mg/l for the food processing industry and 750 mg/l for petroleum refining. Due to potentially harmful osmotic effects on aquatic species and wildlife, the dissolved solids concentration of effluents should not increase the natural background level of the receiving body by more than one third.

### Sample Handling and Preservation

Samples should be collected in glass bottles and refrigerated. Preservatives must not be added.

### Selection of Method

Three methods of determining dissolved solids are available.

Method A, a gravimetric evaporation of a 50 ml aliquot of filtrate is applied to all industrial and sewage samples and to all samples with a conductivity in excess of 450  $\mu\text{S}/\text{cm}$ . It is applied to some samples with conductivities ranging up to 450  $\mu\text{S}/\text{cm}$ .

Method B is an estimation of dissolved solids from the conductivity of the sample. This method is used if dissolved solids is requested, and if the conductivity of the sample is less than 450  $\mu\text{S}/\text{cm}$ .

Method C, although never reported, is an estimate of dissolved solids from the measured values for 10 parameters whenever the appropriate data is available. This calculated estimate is utilized as a quality control check.

Method B and C are not described in detail; however a summary sheet is provided for each.

## SOLIDS-DISSOLVED

### Gravimetric Method A

#### SUMMARY

<b>Matrix.</b>	This method is used routinely on river, lake, sewage and industrial effluent samples.
<b>Substance determined.</b>	The residue from a filtered aliquot of sample which has been evaporated at $103 \pm 2^{\circ}\text{C}$ .
<b>Interpretation of results.</b>	The dissolved solids, which are expressed in mg/l, include ionic species, colloidal matter, and non-ionic substances. Volatile constituents are partially recovered at best.
<b>Principle of method.</b>	A volume of sample is vacuum filtered through pre-washed glass fibre filter paper; a 50 ml aliquot of filtrate is evaporated overnight at $103 \pm 2^{\circ}\text{C}$ , and the dissolved solids calculated in mg/l from the weight of residue.
<b>Time required for analysis.</b>	2 days.
<b>Range of application.</b>	Any samples which is filterable under the specified experimental conditions. Routine working ranges are <1000 mg/l for river and lakes samples and <5000 mg/l for sewage and industrial waste samples.
<b>Standard deviation.</b>	For rivers and lakes in the < 1000 mg/l range, standard deviations are 4.43 for 20-50% of the range and 6.33 for 50-100% of the range. For sewage and industrial wastes in the <5000 mg/l range, standard deviations are 13.5 for 0-20% of the range, 29.6 for 20-50% of the range, and 37.8 for 50-100% of the range.
<b>Accuracy.</b>	<p>The accuracy of the balance is checked daily by weighing appropriate class S weights. For surface waters, the average recovery of 10 and 50 g weights was 99.999% with relative standard deviations ranging from 0.0005 to 0.00014%. For sewage and industrial wastes, the average recovery of 30 and 50 weights was 100.0005% with relative standard deviations ranging from 0.00022 to 0.000108%.</p> <p>Accuracy estimates for dissolved solids methodology are not available.</p>

<b>Detection criteria.</b>	Not yet established.
<b>Interferences and shortcomings.</b>	Some samples are not amenable to filtration while others contain oily substances or acids that inhibit evaporating to a constant weight; volatile constituents are incompletely recovered at best.
<b>Minimum volume of sample.</b>	75 ml.
<b>Preservation and sample container.</b>	Glass bottles are acceptable, and samples should be refrigerated. Preservatives must not be added.
<b>Safety Considerations.</b>	Exhaust system for drying oven must be efficient to protect operator from noxious fumes.



## SOLIDS-DISSOLVED

### Gravimetric Method A

#### 1. Introduction

A portion of sample is vacuum filtered through pre-washed Reeve Angel 934AH glass fibre filter discs; a 50 ml aliquot of filtrate is evaporated overnight at  $103 \pm 2^{\circ}\text{C}$ . Weighings are performed on a balance with 5-decimal place resolution.

#### 2. Interferences and Shortcomings

Some samples cannot be filtered under the specified experimental conditions. Volatile constituents are lost during the evaporation step while the presence of oils, greases, and acids inhibits evaporation to a constant weight.

#### 3. Apparatus

- 3.1. Porcelain evaporating dishes, Coors type, maximum capacity 60 ml.
- 3.2. Glass fibre filter paper discs, Reeve Angel 934AH, 9.0 or 4.95 cm in diameter.
- 3.3. Funnels, Buchner, of appropriate diameter for filter papers and vacuum filtration unit.
- 3.4. Pipettes, 50 ml volumetric, wide-mouthed.
- 3.5. Receiving flasks (for filtrate).
- 3.6. Balance, capable of weighing to 5 decimal places; a 5-place Mettler HL/52 recommended.

The microprocessor-electrobalance system consists of the following modules:

- 5 place Mettler electrobalance HL/52
- digital balance display
- balance control
- computer - Hewlett Packard 9810A
- typewriter interface - Hewlett Packard 11201A
- typewriter - Facit

- 3.7. Class S weights: 10, 30 and 50 g.

#### 4. Reagents

None.

## 5. Procedure

Dissolved and suspended solids are frequently determined concurrently to economize on the technician's time and equipment. The following description, however, only pertains to dissolved solids determinations.

- 5.1. Dry numbered Coors evaporating dishes in oven at  $103 \pm 2^{\circ}\text{C}$ . Cool and store in a desiccator.
- 5.2. Dust interior of balance. Level and zero balance. Weigh class S weights designated A and B; ensure that the A plus B and A minus B values conform to specified limits.
- 5.3. Tare dishes; record weights and dish numbers.
- 5.4. Filter at least 75 ml sample into a clean receiving flask using pre-washed filter papers and suitable vacuum filtration unit.
- 5.5. Pipette 50 ml aliquot of filtrate with wide-mouth volumetric pipette into tared evaporating dish. Evaporate overnight in oven maintained at  $103 \pm 2^{\circ}\text{C}$ ; cool and store in a desiccator.

**NOTE:** Analyze at least 1 out of every 24 samples in duplicate.

- 5.6. Dust, level, and zero balance; repeat A and B quality control procedure.
- 5.7. Weigh evaporating dish plus residue.

### 5.8. Maintenance

- 5.8.1. Ensure that the glassware and evaporating dishes are clean.
- 5.8.2. Evaporating dishes must be discarded as soon as they become chipped or pitted.
- 5.8.3. Keep balance clean at all times.

Follow maintenance procedure outlined in manufacturer's manual, and keep a record of major overhauls, breakdowns, and "remarks".

## 6. Calculation and Reporting

$$\text{Dissolved Solids (mg/l)} = (C - D) \times \frac{10^6}{50}$$

Where:

C = the weight (g) of the Coors dish plus residue.

D = the weight (g) of the Coors dish.

Report results to three significant figures if feasible.

## 7. Precision and Accuracy

Precision is determined by the analysis of within-run duplicates as follows:

Sample	Concentration range (mg/l)	S <sub>ld</sub>	S <sub>md</sub>	S <sub>hd</sub>
Rivers and lakes	< 1000	-	4.43	6.33
Sewage and industrial waste	< 5000	13.5	29.6	37.8

Where:

S<sub>ld</sub> = standard deviation of low-level duplicates (0 - 20% of routine operating range).

S<sub>md</sub> = standard deviation of mid-level duplicates (20 - 50% of routine operating range).

S<sub>hd</sub> = standard deviation of high-level duplicates (50 - 100% of routine operating range).

The accuracy of the balance is checked daily by weighing appropriate class S weights. For surface waters, the average recovery of 10 and 50 g weights was 99.999% with relative standard deviations ranging from 0.0005 to 0.00014%. For sewage and industrial wastes, the average recovery of 30 and 50 weights was 100.0005% with relative standard deviations ranging from 0.00022 to 0.000108%.

Accuracy estimates for dissolved solids methodology are not available.

## 8. Bibliography

- 8.1. American Public Health Association, American Water Works Association and Water Pollution Control Federation (1975). Standard Methods for the Examination of Water and Wastewater. 14th edition. APHA, Washington, D.C. 89.
- 8.2. Ministry of the Environment (1975). Outlines of Analytical Methods. Laboratory Services Branch, Rexdale, Ontario.

## SOLIDS-DISSOLVED

### Calculated from Conductivity Method B

#### SUMMARY

<b>Matrix.</b>	This method is used on rivers, lakes, and drinking waters if the conductivity is less than 450 $\mu\text{S}/\text{cm}$ .
<b>Substance determined.</b>	Dissolved solids as calculated from conductivity measurement.
<b>Interpretation of results.</b>	Results are reported in mg/l. The calculated value assumes that the dissolved solids are ionic species and that the sample was collected from a source relatively free of man-made pollution.
<b>Principle of method.</b>	<p>Conductivity of the sample is measured (refer to Determination of Conductivity for description of method) and for Ontario lakes and rivers, free of industrial pollution, dissolved solids concentration in mg/l is calculated as follows:</p> $\text{Dissolved solids (mg/l)} = 0.65 \times \text{Conductivity in } \mu\text{S/cm}$ <p>provided that conductivity is less than 450 <math>\mu\text{S}/\text{cm}</math> and that the pH of the sample lies between 5 and 9.</p>
<b>Time required for analysis.</b>	5 minutes.
<b>Range of application.</b>	Ontario surface waters relatively free of pollution with conductivities less than 450 $\mu\text{S}/\text{cm}$ and pH values between 5 and 9.
<b>Standard deviation.</b>	2.5 mg/l for dissolved solids range <290 mg/l.
<b>Accuracy.</b>	Limited by validity of conversion factor. See Interferences and Shortcomings below.
<b>Detection Criteria.</b>	0.24 mg/l.
<b>Interferences and shortcomings.</b>	Conversion factor ( $0.65 \pm 0.10$ ) for conductivity to dissolved solids was developed from a large mass of data of rivers and lakes in Ontario.
<b>Minimum volume of sample.</b>	75 ml.

**Preservation and  
sample container.**

Glass bottles should be used and no preservatives should be added.

**Safety  
Considerations**

None.

## SOLIDS-DISSOLVED

### Summation of Ionic Species Method C

#### SUMMARY

<b>Matrix.</b>	This method is used on rivers, lakes, and drinking waters as an in-house quality control check.
<b>Substance determined.</b>	Dissolved solids are calculated from the measured values of 10 parameters.
<b>Interpretation of results.</b>	Results are reported in mg/l. The calculated value assumes that all the dissolved solids arise from these 10 constituents.
<b>Principle of method.</b>	The chemical composition of most surface waters which are relatively free of pollution primarily includes Ca, Mg, K, Na, H, $\text{NH}_4$ , Cl, $\text{NO}_3$ , $\text{SO}_4$ , and $\text{HCO}_3$ ions. On drying, the latter would be converted to $\text{CO}_3$ and H ions. Dissolved solids (mg/l) are then estimated by summation. This value is not reported per se, but is used as a quality control check on sample analyses.
<b>Time required for analysis.</b>	5 minutes, if the data for all parameters are available.
<b>Range of application.</b>	This calculation can be performed on samples in any range; however at least 7 of the foregoing parameters are required; Ca, Mg, Na, K, Cl, $\text{SO}_4$ , and alkalinity (dissolved inorganic carbon).
<b>Standard deviation.</b>	Not available.
<b>Accuracy.</b>	Not available.
<b>Detection criteria.</b>	Not available.
<b>Interferences and shortcomings.</b>	Less common elements which are presumed to be present in small concentrations are not included in the calculation. This procedure is only practical when detailed analytical data are already available.
<b>Minimum volume of sample.</b>	1000 ml.
<b>Preservation and sample container.</b>	Glass bottles should be used. No preservatives should be added.
<b>Safety Considerations.</b>	None.

## THE DETERMINATION OF SOLIDS-IGNITED

The ignited solids test determines both the quantity of material that remains as a residue and the quantity of material that is lost when the total, suspended, or dissolved solids residue is ignited at  $600^{\circ}\text{C}$  for one hour. The test does not determine specific substances but only a general class of substances which have a similar response under the same test conditions.

The loss on ignition is due to oxidation of carbon and hydrogen, escape of water of hydration, volatilization of inorganic ammonium salts, decomposition of magnesium carbonate, and loss of any other materials which are volatile or decomposable below  $600^{\circ}\text{C}$ . Thus, the loss on ignition generally over estimates the organic content. The actual organic content is lower by the amount of water, inorganic salts, etc., released. In the case of clean natural waters, the loss on ignition is due almost entirely to non-carbonaceous material; therefore caution should be exercised in interpreting the results on such samples.

Ignited solids results are most useful in the characterization of sewage sludges where it is desirable to estimate the organic fraction. It is one of the major tests used in assessing the efficiency of sludge digestion. This test is rarely done on clean water samples.

### Sample Handling and Preservation

Samples may be collected in glass bottles and refrigerated. Do not add preservatives, freeze, or fill to the brim if the sample is a sludge.

### Selection of Method

Ignited solids are determined gravimetrically by igniting the total, suspended, or dissolved solids residue at  $600 \pm 10^{\circ}\text{C}$  for 1 hour.

**SOLIDS-IGNITED**  
**Gravimetric Method A**

**SUMMARY**

<b>Matrix.</b>	This method is performed on total suspended or dissolved solids from sewage and industrial waste samples.
<b>Substance determined.</b>	The ignited solids test determines both the quantity of material that remains as a residue and the quantity of ash that remains when the total, suspended, or dissolved solids are ignited at $600 \pm 10^{\circ}$ C for one hour. The quantity of volatile material is calculated, i.e., the difference.
<b>Interpretation of results.</b>	<p>Results are reported in mg/l. The volume utilized in all calculations is the starting aliquot of sample. The dried residue source is identified (total, suspended or dissolved) and both the loss on ignition and residual ash concentrations are reported.</p> <p>This test is primarily performed on sewage streams to estimate volatile organic content; when applied to "clean" samples, the loss on ignition is basically non-carbonaceous.</p>
<b>Principle of method.</b>	Analysis is accomplished gravimetrically by igniting the total, suspended, or dissolved solids residue at $600 \pm 10^{\circ}$ C for 1 hour.
<b>Time required for analysis.</b>	4 hours, if the total, suspended or dissolved solids data and residues are available.
<b>Range of application.</b>	Any range.
<b>Standard deviation.</b>	For sewage and industrial waste samples in the < 1000 mg/l range standard deviations for determination of the ash are 10.14 for 0 - 20% of the range; 21.6 for 20 - 50% of the range and 14.4 for 50 - 100% of the range. For samples in the < 50,000 mg/l range, standard deviations are 19.6 for 0 - 20% of the range, 109 for 20 - 50% of the range and 354 for 50 - 100% of the range. Data are based on within-run duplicates.
<b>Accuracy.</b>	The accuracy of the balance is checked daily by weighing appropriate class S weights. The average recovery of 30 and 50 g weights was 100.0005% with relative standard deviations ranging from 0.00022 to 0.000108%. Accuracy estimates for ignited solids methodology are not available.



<b>Detection criteria.</b>	Not yet established.
<b>Interferences and shortcomings.</b>	The inaccuracy and imprecision associated with the suspended, dissolved or total solids procedures are included in the ignition step. Loss on ignition cannot be attributed solely to one parameter since a variety of elements and materials are volatilized during ignition.
<b>Minimum volume of sample.</b>	300 ml.
<b>Preservation and sample container.</b>	Glass bottles are adequate, but must not be filled to the brim for sludges. Preservatives should not be added, but the sample should be refrigerated.
<b>Safety considerations.</b>	Use asbestos gloves and crucible tongs when removing samples from the muffle furnace. The crucibles require about 1 hour to cool to room temperature. Exhaust system for muffle furnace must be efficient to protect staff from noxious fumes.

## SOLIDS-IGNITED

### Gravimetric Method A

#### 1. Introduction

The suspended, dissolved, or total solids residue is ignited at  $600^{\circ}\text{C}$  for 1 hour. Both the ash residual and loss on ignition are calculated, and reported as weight (mg) per liter with the calculations being based on the original aliquot of sample (ml). Some samples, (i.e. sludges when analyzed for total ignited solids) cannot be pipetted and are usually scooped into an evaporating dish and weighed prior to drying. The sample is then analyzed in the normal manner, but the result will be reported as mg/kg wet weight.

#### 2. Interferences and Shortcomings

Any problems associated with the total, suspended, and dissolved solids determinations also affect the ignited solids test. Errors resulting from physical losses due to decrepitation are also a possibility.

#### 3. Apparatus

- 3.1. Muffle furnace, capable of maintaining  $600 \pm 10^{\circ}\text{C}$ .
- 3.2. Balance, capable of 5-decimal point resolution.
- 3.3. Petrie dishes, 9.3 cm I.D., pyrex glass.
- 3.4. Desiccators.
- 3.5. All equipment for suspended, dissolved or total solids tests is also required. (See appropriate test method.)

#### 4. Reagents

None.

#### 5. Procedure

THE DRIED RESIDUE FROM THE SUSPENDED, DISSOLVED OR TOTAL TEST PROCEDURE IS REQUIRED (PLEASE SEE THE DETERMINATION OF SUSPENDED, TOTAL AND DISSOLVED SOLIDS). THE SAMPLE ALIQUOT AND DRIED WEIGHT DATA ARE ALSO NECESSARY.

- 5.1. Place dried residue in muffle and wait for temperature to regain  $600 \pm 10^{\circ}\text{C}$ ; ignite for 1 hour. Glass fibre filter paper discs are kept in pyrex glass petrie

dishes rather than racks so they can be heated to 600°C. After firing, cool evaporating dishes and petrie dishes slightly and transfer to desiccators as soon as feasible. Cool to room temperature before weighing. Analyze at least 4% of the samples in duplicate.

- 5.2. Dust, level and zero balance.
- 5.3. Weigh quality controls A and B (Class S weights). Ensure that A plus B and A minus B values are within specified limits.
- 5.4. Weigh ignited samples.
- 5.5. Utilizing the appropriate data, calculate both the residual ash and weight loss due to ignition.

## 6. Calculation and Reporting

- 6.1. Routine type samples, based on sample volume.

$$\text{Loss on ignition (mg/l): } \frac{(A - B)}{V} \times 10^6$$

Where:

B = the weight (g) of ash plus filter paper or evaporating dish after ignition.

A = the weight (g) of residue plus filter paper or dish before ignition.

V = the aliquot of sample (ml) taken for the suspended, dissolved, or total solid determination.

$$\text{Ash (mg/l): } \frac{(B - C)}{V} \times 10^6$$

Where:

C = the weight (g) of the filter paper or evaporating dish.

B = the weight (g) of the ash plus filter paper or evaporating dish.

V = the aliquot of sample (ml) taken for the suspended, dissolved, or total solids determination.

- 6.2. For total ignited solids on sludges only, based on sample weight.

$$\text{Dried weight (mg/l): } \frac{(F - G)}{E} \times 10^6$$

Where:

E = the weight (g) of the wet sample\* plus dish, minus the dish tare.

F = the weight (g) of the dried sample plus dish.

G = the weight (g) of the dish.

$$\text{Loss on ignition (mg/l): } \frac{(F - H)}{E} \times 10^6$$

Where:

E = the weight (g) of the wet sample\* plus dish, minus the dish tare.

F = the weight (g) of the dried sample plus dish.

H = the weight (g) of the ash plus dish.

$$\text{Ash (mg/l): } \frac{(H - G)}{E} \times 10^6$$

Where:

E = the weight (g) of the wet sample\* plus dish, minus the dish tare.

G = the weight (g) of the dish.

H = weight (g) of the ash plus dish.

\* ASSUME DENSITY IS 1 mg/ml.

Results are reported to three significant figures if feasible.

## 7. Precision and Accuracy

For sewage and industrial waste precision is based on the ash of within-run duplicate samples and is as follows:

Sample	Concentration range (mg/l)	S <sub>ld</sub>	S <sub>md</sub>	S <sub>hd</sub>
Suspended solids	<1,000	10.14	21.6	14.4
Total solids	<50,000	19.6	109	354

Where:

S<sub>ld</sub> = standard deviation of low-level duplicate (0 - 20% of routine operating range)

S<sub>md</sub> = standard deviation of mid-level duplicates (20 - 50% of routine operating range)

S<sub>hd</sub> = standard deviation of high-level duplicates (50 - 100% of routine operating range)

The accuracy of the balance is checked daily by weighing appropriate class S weights. The average recovery of 30 and 50 g weights was 100.0005% with relative standard deviations ranging from 0.00022 to 0.000108%. Accuracy estimates for ignited solids methodology are not available.

## 8. Bibliography

8.1. American Public Health Association, American Water Works Association and Water Pollution Control Federation (1975). Standard Methods for the Examination of Water and Wastewater. 14th edition. APHA, Washington, D.C. 89.

8.2. Ministry of the Environment (1975). Outline of Analytical Methods. Laboratory Services Branch, Rexdale, Ontario.

## THE DETERMINATION OF SOLIDS - SUSPENDED

Suspended solids pertain to the material which may be removed from a water sample by filtration. The test does not determine specific chemicals but rather a general class of substances having a similar response to the selected test conditions.

Although suspended solids in waters occur naturally from the life cycle, disturbance of fines in river bottoms, and soil leaching by rainwater, they are too frequently caused by man's mishandling of his environment and by industrial activity. In order to limit the degradation of our water resources, effluents from industrial and waste treatment plants must not contain suspended solids in excess of 15 mg/l. Suspended solids, regardless of origin, have a deleterious effect on many industrial processes, and therefore, maximums are set at 5 mg/l for the textile industry, 10 mg/l for food processing, petroleum refineries and pulp and paper producers and 15 mg/l for the manufacturing of chemicals.

Suspended solids are extremely important in monitoring the operation of a waste treatment plant. The data are required to estimate the loadings applied to aeration tanks. In treating domestic wastes, an efficient water pollution control plant will reduce the suspended solids from 200 to < 15 mg/l.

### Sample Handling and Preservation

Samples are collected in glass bottles and preserved by refrigeration; no chemical preservatives should be added. Ideally a suitable volume of sample should be submitted in a separate container marked "suspended solids only"; in this way laboratory sampling errors are minimized.

### Selection of Method

Suspended solids are determined gravimetrically on the non-filterable residue of a sample aliquot.

## SOLIDS - SUSPENDED

### Gravimetric Method

#### SUMMARY

Matrix.	Suspended solids are routinely determined by this method on river, lake, sewage, and industrial waste samples.
Substance determined.	The non-filterable residue from a sample aliquot.
Interpretation of results.	Suspended solids are reported in mg/l.
Principle of method.	An aliquot of sample (25-500 ml) is vacuum filtered through pre-weighed Reeve Angel 934 AH glass fibre filter paper. The residue is dried at $103 \pm 2^\circ\text{C}$ .
Time required for analysis.	2 days. In this period 100 samples can be analyzed.
Range of application.	Any sample that is filterable under the selected experimental conditions. Normal working ranges are: 0.5-10 mg/l for river and lake samples and 3-15 mg/l, < 500 mg/l and < 5000 mg/l for sewage and industrial waste samples.
Standard deviation.	<p>Rivers and lakes (0.5 - 10.0 mg/l range): 0.299 for 0-20% of the range; 0.364 for 20-50% of the range and 0.481 for 50-100% of the range.</p> <p>Sewage and industrial wastes (&lt; 500 mg/l range): 3.97 for 0-20% of the range; 4.71 for 20-50% of the range and 7.05 for 50-100% of the range.</p>
Accuracy.	<p>The accuracy of the balance is checked by weighing two appropriate class S weights daily. For surface waters, the average recovery of 20 and 100 mg weights is 99.74% with relative standard deviations ranging from 0.008 to 0.050%. For sewage and industrial wastes, the average recovery of 50 and 500 mg weights is 100.11% with relative standard deviations ranging from 0.008 to 0.011%.</p> <p>Accuracy estimates for suspended solids methodology are not available.</p>

**Detection  
criteria.**

Rivers and lakes: 0.492 mg/l.  
Sewage and industrial wastes: 6.53 mg/l.

**Interferences  
and shortcomings.**

Selecting a representative aliquot is a major problem - particularly in sewages where the size of the floc particles varies significantly. Grease and oils, if present, adhere to equipment and adversely affect the drying step.

**Minimum volume  
of sample.**

200 ml for most samples; however, 500 ml is required for suspended sediment determination in the 3-15 mg/l range for sewage and industrial waste samples, and for some river and lake samples.

**Preservation and  
sample container.**

Samples should be collected in glass bottles and refrigerated but not frozen. Preservatives should not be added.

**Safety  
considerations.**

Exhaust system for drying ovens must be efficient to protect operator.

## SOLIDS - SUSPENDED

### Gravimetric Method

#### 1. Introduction

An aliquot of sample is vacuum filtered through pre-washed and pre-weighed Reeve Angel 934AH glass fibre filter papers. The nonfilterable portion is evaporated overnight at  $103 \pm 2^{\circ}\text{C}$ , and the weight of residue measured on a balance capable of five or six decimal point resolution.

#### 2. Interferences and Shortcomings

Some samples cannot be filtered under the specified experimental conditions while the presence of oils and greases inhibits evaporation to a constant weight. Selecting a representative sample is particularly difficult when the size of particulates varies considerably, as is usually the case with sewage samples.

#### 3. Apparatus

- 3.1. Reeve Angel 934AH glass fibre filter paper discs, 9.0 or 4.25 cm in diameter.
- 3.2. Buchner funnels of appropriate diameter for the filter papers and vacuum filtration unit.
- 3.3. Cylinders, 50 to 500 ml capacity; wide mouth volumetric pipettes (25 and 50 ml capacity).
- 3.4. Receiving flasks (for filtrate).
- 3.5. Balance, Sartorius 1802, 6 place modified balance, Mettler, 5-place electrobalance HL52 or an equivalent balance with a minimum resolution of 5 decimal places.

A microprocessor controlled electrobalance system is currently used by the Sewage laboratory and consists of the following modules:

5-place Mettler electrobalance - HL52  
digital balance display BA28  
balance control BE 20  
computer - Hewlett Packard 9810A  
typewriter interface - Hewlett Packard 11201A  
typewriter - Facit

- 3.6. Weights (Class S), 100 mg and 20 mg for surface waters; 500 mg and 50 mg for sewages and industrial wastes (for QC-A and QC-B).



#### 4. Reagents

None.

#### 5. Procedure

Dissolved and suspended solids are frequently determined concurrently, but the following description pertains to suspended solids only.

5.1. Prewash filter papers with distilled water; dry thoroughly in an oven at  $103 \pm 2^{\circ}\text{C}$ , and cool in a desiccator. Prewashed papers need not be stored in a desiccator. Filter paper discs 4.25 cm in diameter are normally used for river and lake samples, while 9.0 cm discs are used for sewage and industrial waste samples.

5.2. Dust interior of balance; level and zero balance. Weigh QC-A and QC-B weights and record. Ensure that (A+B) and (A-B) fall within specified control limits.

5.3. Tare pre-washed and pre-dried filter papers; record weights maintaining order of filter papers. Although any number of samples can be analyzed at a time, samples are usually handled in multiples of 25 - particularly for the electro-balance system.

5.4. Select an appropriate aliquot for the sample and record the volume:

5.4.1. If a sample is submitted in a glass bottle designated "for suspended solids only", the entire volume of sample is vacuum filtered. The actual volume in the container is measured using a cylinder; both the sample container and the cylinder are thoroughly rinsed with distilled water and the washings included in the filtration step. This sampling technique was utilized for the Pluarg program where samples were submitted in containers similar to milk bottles but equipped with plastic snap-on lids; the container capacity was approximately 450 ml.

5.4.2. The normal aliquot, for samples submitted for multiple tests, is 200 ml. The volume is measured with a cylinder; the cylinder is then rinsed with approximately 50 ml of distilled water, and the washings are included in the vacuum filtration step. (When suspended and dissolved solids are determined concurrently, the aliquot for the dissolved solids test is pipetted before the washings are added.) The selected aliquot volume may be varied according to the quantity of particulates, but the minimum aliquot is 25 ml (a few sludges) and the maximum is 500 ml (rivers, lakes, or storm sewers).

**NOTE:** Where sample volume permits, analyze at least one out of every 24 samples in duplicate.

5.5. The filter papers plus nonfilterable matter are dried at  $103 \pm 2^{\circ}\text{C}$  overnight on racks or perforated plates. Cool and store in a desiccator until weighed.

5.6. Dust, level, and zero balance. Repeat A and B quality control procedure.

5.7. Weigh dried filter papers plus residue and record weights. The recording is unnecessary for the electrobalance system, but the order of the filter papers must be maintained for both the automated and manual systems.

#### 5.8. Maintenance

5.8.1. Ensure that all glassware, filtration units, filter paper racks and perforated plates are clean and free of oily deposits.

5.8.2. Keep balance clean at all times. Follow the maintenance procedure outlined in the manufacturer's manual, and keep a record of major overhauls, breakdowns, and remarks.

### 6. Calculation and Reporting

$$\text{Suspended Solids (mg/l)} = \frac{(c - d) 10^6}{v}$$

Where:

c = weight (g) of the filter paper plus dried nonfilterable material

d = weight (g) of the filter paper

v = volume (ml) of sample filtered

Report to three significant figures if feasible.

\* If the selected balance displays weights in mg, the power index is 3.

### 7. Precision and Accuracy

Precision is determined by the analysis of withinrun duplicates as follows:

Sample	Concentration range (mg/l)	$S_{ld}$	$S_{md}$	$S_{hd}$
River and lakes	0.5 - 10.0	0.299	0.364	0.481
Sewage and industrial wastes	< 500	3.97	4.71	7.05

Where:

$S_{ld}$  = standard deviation of low-level duplicates (0-20% routine operating range)

$S_{md}$  = standard deviation of mid-level duplicates (20-50% routine operating range)

$S_{hd}$  = standard deviation of high-level duplicates (50-100% routine operating range)

The accuracy of the balance is checked by weighing two appropriate class S weights daily. For surface waters, the average recovery of 20 and 100 mg weights is 99.74% with relative standard deviations ranging from 0.008 to 0.050%. For sewage and industrial wastes, the average recovery of 50 and 500 mg weights is 100.11% with relative standard deviations ranging from 0.00 to 0.011%. Accuracy estimates for suspended solids methodology are not available.

## 8. Bibliography

- 8.1. American Public Health Association, American Water Works Association and Water Pollution Control Federation (1975). Standard Methods for the Examination of Water and Wastewater. 14th edition. APHA, Washington, D.C. 89.
- 8.2. Ministry of the Environment (1975). Outlines of Analytical Methods. Laboratory Services Branch, Rexdale, Ontario.

## THE DETERMINATION OF SOLIDS - TOTAL

Total solids refers to the quantity of material that remains as a residue after the water is removed from a sample by evaporation at  $103 \pm 2^{\circ}\text{C}$ . The test does not determine specific chemical substances, but examines only a general class of substances which have a similar response under the same test conditions. Total solids are normally estimated from suspended and dissolved solids data; gravimetric determinations are only performed on samples which are not amenable to filtration. The separation of the solids contents into dissolved and suspended fractions is considered preferable for environmental studies and the efficient operation of water treatment plants; see appropriate test procedures for the significance of dissolved and suspended solids measurements.

### Sample Handling and Preservation

Samples may be collected in glass bottles and refrigerated. No preservatives should be added.

### Selection of Method

Total solids are normally estimated from suspended and dissolved solids data, Method A. The gravimetric procedure (Method B) is available on request or for samples that are not amenable to filtration. The latter are usually digester sludges from sewage plants.

**SOLIDS -TOTAL**  
**Calculation Method A**

**SUMMARY**

<b>Matrix.</b>	This method is the preferred method for rivers and lakes samples, sewage, and industrial waste.
<b>Substance determined.</b>	Total solids concentration.
<b>Interpretation of results.</b>	Results are reported in mg/l.
<b>Principle of method.</b>	Addition of suspended and dissolved solids concentrations.
<b>Time required for analysis.</b>	10 seconds.
<b>Range of application.</b>	Any sample which is amenable to filtration under the selected experimental conditions.
<b>Standard deviation.</b>	See procedures for suspended and dissolved solids.
<b>Accuracy.</b>	Not applicable.
<b>Detection criteria.</b>	See procedures for suspended and dissolved solids.
<b>Interferences and shortcomings.</b>	The defects of the suspended and dissolved solids tests are combined.
<b>Minimum volume of sample.</b>	300 ml.
<b>Preservation and sample container.</b>	Samples may be collected in glass containers, and should be refrigerated. Preservatives should not be added.
<b>Safety considerations.</b>	None.

## SOLIDS -TOTAL

### Gravimetric Method B

#### SUMMARY

<b>Matrix.</b>	This method is usually performed on sewage and industrial waste samples which are not amenable to filtration and contain a high concentration of suspended matter.
<b>Substance determined.</b>	The residue from a 50 ml aliquot of sample that has been dried overnight at $103 \pm 2^{\circ}\text{C}$ .
<b>Interpretation of results.</b>	Results are reported in mg/l.
<b>Principle of method.</b>	An aliquot of sample is dried to a constant weight by heating at $103 \pm 2^{\circ}\text{C}$ .
<b>Time required for analysis.</b>	2 days.
<b>Range of application.</b>	Although the test can be applied to any water sample, it is normally restricted to samples with a high portion of suspended matter.
<b>Standard deviation.</b>	For the <400,000 mg/l range, standard deviations are 200 for 0 - 20% of range, 400 for 20 - 50% of range and 1000 for 50 - 100% of range based on within-run duplicate analyses.
<b>Accuracy.</b>	<p>The accuracy of the balance is checked by weighing appropriate class S weights. For surface waters, the average recovery of 10 and 50 g weights was 99.999% with relative standard deviations ranging from 0.0005 to 0.00014%. For sewage and industrial wastes, the average recovery of 30 and 50 g weights was 100.0005% with relative standard deviations ranging from 0.00022 to 0.000108%.</p> <p>Accuracy estimates for total solids methodology are not available.</p>
<b>Detection criteria.</b>	Not yet established.
<b>Interferences and shortcomings.</b>	As the test is applied to samples with a high concentration of suspended matter, the selection of a representative aliquot is the principle source of error. Volatile matter is only partially recovered.

**Minimum volume  
of sample.**

75 ml.

**Preservation and  
sample container.**

Samples may be collected in glass or plastic containers, but the container must be only half filled if it is a sludge sample. The samples should be refrigerated but preservatives must not be added.

**Safety  
considerations.**

Exhaust system for drying ovens must be efficient to protect operator from noxious fumes.

**SOLIDS -TOTAL**  
**Gravimetric Method B**

**1. Introduction**

A 50 ml aliquot of sample is dried overnight at  $103 \pm 2^{\circ}\text{C}$ . The amount of dried residue is determined and the results reported in mg/l.

**2. Interferences and Shortcomings**

Total solids determinations are normally restricted to samples with a high portion of suspended material and thus selecting a representative aliquot is the principle difficulty. The presence of grease and oils may inhibit drying to a constant weight at  $103^{\circ}\text{C}$ , and volatile constituents will be lost. Similar drying difficulties may arise from samples with extremely low pH values.

**3. Apparatus**

- 3.1. Evaporating dishes, Coors ceramic, approximately 60 ml capacity.
- 3.2. Desiccators.
- 3.3. Pipettes, wide mouth 50 ml capacity.
- 3.4. Oven capable of maintaining  $103 \pm 2^{\circ}\text{C}$ .
- 3.5. Balance, 5-decimal place resolution.
- 3.6. Class S weights: 10, 30, and 50 g.

**4. Reagents**

None

**5. Procedure**

- 5.1. Dry numbered, Coors evaporating dishes overnight in an oven at  $103 \pm 2^{\circ}\text{C}$ . Cool in a desiccator.
- 5.2. Dust, level, and zero balance. Weigh class S weights designated A and B. Ensure that the A plus B and A minus B values are within the specified limits.
- 5.3. Weigh dried evaporating dishes, and record weights and identification.
- 5.4. Pipette a 50 ml aliquot of sample into the Coors dish. If pipetting is not practical, pour approximately 50 ml sample to the dish and obtain the wet



weight. The result is reported as mg/kg wet weight.

- 5.5. Dry crucible plus sample overnight at  $103 \pm 2^\circ\text{C}$ . Cool in a desiccator.
- 5.6. Dust, level, and zero balance. Repeat quality control A and B test described above.
- 5.7. Weigh crucibles plus dried residue; record weight.

## 6. Calculation and Reporting

$$\text{Total Solids (mg/l)} = \frac{C - D}{V} \times 10^6$$

Where:

C = weight (g) of evaporating dish plus dried residue.

D = weight (g) of evaporating dish.

V = volume (ml) or wet weight (g) of sample.

## 7. Precision and Accuracy

Based on within-run duplicates, the standard deviations for the less than 400,000 mg/l range are 200 for 0 - 20% of the range, 400 for 20 - 50% of the range and 1000 for 50 - 100% of the range.

The accuracy of the balance is checked by weighing appropriate class S weights. For surface waters, the average recovery of 10 and 50 g weights was 99.999% with relative standard deviations ranging from 0.0005 to 0.00014%. For sewage and industrial wastes, the average recovery of 30 and 50 g weights was 100.0005% with relative standard deviations ranging from 0.00022 to 0.000108%.

Accuracy estimates for total solids methodology are not available.

## 8. Bibliography

- 8.1. American Public Health Association, American Water Works Association and Water Pollution Control Federation (1975). Standard Methods for the Examination of Water and Wastewater. 14th edition. APHA, Washington, D.C. 89.
- 8.2. Ministry of the Environment (1975). Outlines of Analytical Methods. Laboratory Services Branch, Rexdale, Ontario.

## THE DETERMINATION OF SULPHATE

Sulphates are widely distributed in the natural environment, occurring in sedimentary rocks such as gypsum ( $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$ ) and in the upper aerobic layers of soil where sulphides are converted to sulphates. Plant and animal decomposition contribute sulphates to the environment since sulphur is a major plant nutrient and plays an integral role in animal life processes.

Most sulphates are highly soluble and are widely distributed in natural waters, usually in low concentrations. High sulphate levels, however, are often present in mine drainage waters as a result of pyrite oxidation. Sodium and magnesium sulphate exert a cathartic effect on man and livestock. Consequently, a 250 mg/l sulphate limit for potable water has been set by the Ontario Ministry of the Environment.

Sulphate in soils and sediments occurs in soil minerals such as gypsum and pyrite, and also in the readily extractable, water soluble form and in the adsorbed state. Adsorption occurs principally on iron and aluminum coatings, and phosphates, and depends on pH.

Sulphates are present in the atmosphere and are significantly increased by coal combustion which is used in many industrial activities. Sulphur dioxide and trioxide emissions to the atmosphere are dissolved by rainwater and returned to the earth's surface as dilute sulphurous and sulphuric acids. The former are readily oxidized in the air to sulphuric acid. This acid precipitation may have detrimental effects on vegetation and aquatic life and corrosive effects on man-made structures. The desirable ambient air quality criterion for sulphation rate of the atmosphere in Ontario is  $0.7 \text{ mg SO}_3/100 \text{ cm}^2/\text{day}$ , as set by the Environmental Protection Act, 1974.

### Sample Handling and Preservation

#### Water, Sewage and Industrial Waste, and Precipitation

Although most samples received for sulphate analysis are quite stable, some samples may contain bacteria which can reduce sulphate to sulphide. Samples suspected of containing such bacteria should be cooled and kept refrigerated prior to analysis. Sulphite (usually only present in industrial wastes samples) may be oxidized to sulphate by dissolved oxygen above a pH of 8.0. Samples containing sulphite should have the pH adjusted to below this level.

#### Soil and Sediments

Samples of approximately 50 g are recommended and are collected in glass or plastic jars. Samples require no preservative and upon arrival at the laboratory are air dried and ground.

### Air Particulates

Samples are collected on quartz or glass fibre Hi-Vol filters by means of a high volume air sampling machine. Filters are folded and placed in envelopes for transport to the laboratory. No preservatives are required. Plastic gloves should be worn when handling the filters to prevent contamination.

### Ambient Air

Care should be taken when removing the candle from the shelter at the sampling site so that the lead oxide coating is not lost or damaged. Unnecessary jarring of instrument during transportation back to the lab should be avoided.

### Dustfall

Dustfall samples are collected in jars as described in The Determination of Dustfall. The water soluble fraction is used for sulphate measurement.

### **Selection of Method**

Method A, a methylthymol blue automated colorimetric method is currently used for sulphate determinations on drinking water, river, lake, sewage and industrial effluent samples, Hi-Vol extracts and occasionally on sediment extracts. This method is rapid and precise. Method B, an automated ion chromatographic procedure is used on precipitation, surface water and some air particulate samples and simultaneously determines sulphate, chloride, and nitrate. This method is rapid and encounters little interference.

## SULPHATE

### Methylthymol Blue Automated Colorimetry Method A

#### SUMMARY

<b>Matrix</b>	This method is applied to drinking water, river, lake, sewage and industrial effluent samples, dustfall, Hi-Vol extracts and occasionally on sediment samples.
<b>Substance determined.</b>	Sulphate ion, $\text{SO}_4^{=}$ .
<b>Interpretation of results.</b>	Results are reported as mg/l $\text{SO}_4$ . Sulphate is a common mineral component of all natural waters. Its concentration normally varies between 10 and 80 mg/l, but may be as high as several thousand mg/l in some industrial discharges or in drainage from pyrite mine tailings. Rainfall may contain perceptible amounts of sulphate if local industrial processes are emitting sulphur dioxide and sulphur trioxide to the atmosphere.
<b>Principle of method.</b>	Samples are automatically pretreated by ion exchange to remove cationic interference, and then reacted with a barium methylthymol blue complex to produce barium sulphate and free methylthymol blue. The concentration of the free methylthymol blue, which is directly proportional to the sulphate concentration, is then determined colorimetrically at 460 nm.
<b>Time required for analysis.</b>	Approximately 10 - 15 analyses can be performed in an hour. Operation of the AutoAnalyzer equipment can be maintained for as long as 24 hours a day, performing upwards of 300 tests.
<b>Range of application.</b>	Routine operational ranges are from 1 - 50 mg/l for river and lake samples (AAII system) and 1 - 100 mg/l for drinking water, sewage and industrial waste samples and occasionally sediment and Hi-Vol extracts (AAI system).
<b>Standard deviation.</b>	For the 1 - 50 mg/l range, the standard deviations are 0.420 for 0 - 20% of the range; 0.536 for 20 - 50% of the range and 0.673 for 50 - 100% of the range. For the 1 - 100 mg/l range standard deviations are 0.539 for 0 - 20% of the range; 0.59 for 20 - 50% of the range and 0.57 for 50 - 100% of the range.
<b>Accuracy.</b>	For the AAI system accuracy is controlled by 2 independently prepared long-term standards (QC-A and QC-B) such that (A + B) and (A - B) do not vary by more than 3 mg/l (3 standard deviations) from their long-term means.

For the AAI system (1 - 50 mg/l range) accuracy is controlled in a similar way with control limits of 1.43 mg/l.

**Detection  
criteria.**

0.69 for the 1 - 50 mg/l range. 0.89 for the 1 - 100 mg/l range.

**Interferences and  
shortcomings.**

Cations such as calcium, magnesium and other heavy metals are removed by ion exchange to avoid large negative interference effects. Sulphide in excess of 5 mg/l, sulphite in excess of 1.0 mg/l, phosphate in excess of 0.8 mg/l and tannic acid in excess of 20 mg/l all produce positive interference effects. Interferences may occur when analyzing samples with pH's significantly above or below 7 and/or with high conductivities, since these conditions adversely affect the performance of the ion exchange column. Naturally colored substances absorbing near 460 nm result in a positive interference.

**Minimum volume  
of sample.**

50 ml.

**Preservation and  
sample container.**

Glass or plastic bottles are acceptable for liquid samples. Heavily polluted samples should be stored at low temperatures to prevent bacterial reduction of sulphate to sulphide. Sediments are air dried, ground and stored in glass or plastic jars. No preservative is required. Hi-Vol filters are stored in envelopes for transport to the laboratory.

**Safety  
considerations.**

Barium chloride is extremely toxic. Both the crystals and solutions should not be allowed to contact the skin. Caution should be exercised in handling concentrated hydrochloric acid and sodium hydroxide as they can cause severe burns.

## SULPHATE

### Methylthymol Blue Automated Colorimetry Method A

#### 1. Introduction

A portion of sample enters the AutoAnalyzer system where it is successively diluted by a factor of 4.3 (greater dilutions may be required for Hi-Vol and sediment extracts) and then passed through a glass column containing Amberlite IR-120 cationic exchange resin. This removes calcium and magnesium both of which cause large negative interferences. The pretreated sample is mixed with a color reagent containing equimolar quantities of methylthymol blue (MTB) and barium ions in an aqueous ethanol solution adjusted to a pH between 2.5 and 3.0 with 1N hydrochloric acid.

When a water sample containing sulphate reacts with this reagent, barium sulphate is produced. After allowing sufficient time for the production of barium sulphate, the pH is re-adjusted to between 12.3 and 13.0 with 0.18N sodium hydroxide. At this high pH, any barium ions remaining in solution complex with available methylthymol blue leaving an amount of uncomplexed methylthymol blue in solution which is equivalent to the quantity of sulphate removed as barium sulphate. The uncomplexed methylthymol blue is then determined colorimetrically in a 5 cm flow cell at 460 nm and monitored on a chart recorder. The result in mg/l sulphate is determined from the chart recorder trace by comparison with peaks produced by a similarly treated set of standards. The AAI system is presently used for the analysis of drinking water, sewage and industrial waste samples and the AAI system is used for river and lake samples, Hi-Vol and sediment extracts. Soil and sediments are first extracted with 0.01 M calcium chloride.

#### 2. Interferences and Shortcomings

Calcium and magnesium are the most frequently encountered interferences in natural waters. These and most other metallic cations which are common constituents of natural waters will interfere by complexing with the methylthymol blue dye in much the same way as barium.

Calcium and magnesium removal by cation exchange is essential to the satisfactory performance of the methylthymol blue method under routine operating conditions.

Sulphide in excess of 5 mg/l, sulphite in excess of 1.0 mg/l, phosphate in excess of 0.8 mg/l and tannic acid in excess of 20 mg/l all produce known positive interference effects, which cannot presently be avoided.

Samples with pH's significantly different from pH 7, and with conductivities exceeding 3,000  $\mu$ mhos/cm may cause the release of interfering substances from the cation exchange column, producing a large negative interference particularly if the predominant cation in solution is sodium.

Natural sample color causing an absorbance in the yellow region near 460 nm is a potential positive interference.

### 3. Apparatus

- 3.1. AutoAnalyzer (Technicon or equivalent) system with the following modules:
  - 3.1.1. sampler.
  - 3.1.2. proportioning pump.
  - 3.1.3. colorimeter equipped with 460 nm filters and 5.0 cm flow cell.
  - 3.1.4. voltage regulator.
  - 3.1.5. chart recorder.
- 3.2. Pump tubing manifold and associated manifold glassware as shown in Figure 1 for AAI and Figure 2 for AAII systems.
- 3.3. Culture tubes, 19 x 150 mm.
- 3.4. Culture tube racks with a 40 tube capacity.
- 3.5. Ion exchange column constructed from a 23 cm glass tube with an internal diameter of 3 mm. The tube is packed with Amberlite IR-120 cationic exchange resin and plugged on both ends with glass wool.
- 3.6. Reagent bottles, glass, 1 liter (20).
- 3.7. Reagent reservoir bottles, 1 liter (2), 4 liter (1).

#### For Hi-Vol Filter Extraction Only

- 3.8. Beakers, 100 ml.
- 3.9. Hot plate, thermostatically controlled.
- 3.10. Graduated cylinders, 100 ml.
- 3.11. Filter paper, Whatman No. 40, 11 cm diameter.
- 3.12. Funnels, appropriate for 11 cm diameter filters.

#### For Soil and Sediment Extraction Only

- 3.13. Erlenmeyer flasks, 125 ml.
- 3.14. Filter assembly, Whatman No. 30.
- 3.15. Tubes, 50 ml.
- 3.16. Shaker, horizontal.

#### 4. Reagents

- 4.1. Methylthymol blue (also known as Methylthymol blue complexone). Chemical formula 3', 3'' Bis N, N-bis (carboxymethyl)-aminol-methyl thymolsulphon-phthalein pentasodium salt ( $C_{37}H_{39}N_2Na_5O_{13}S$ ); molecular weight 866.73, Eastman Cat. No. 8068, reagent grade powder.
- 4.2. Barium chloride dihydrate ( $BaCl_2 \cdot 2H_2O$ ), reagent grade crystals.
- 4.3. Sodium hydroxide (NaOH), reagent grade pellets.
- 4.4. Hydrochloric acid (HCl), reagent grade, concentrated.
- 4.5. Ethanol ( $C_2H_5OH$ ), reagent grade (95%).
- 4.6. Sodium sulphate ( $Na_2SO_4$ ), anhydrous, reagent grade.
- 4.7. Amberlite IR-120 or equivalent cationic exchange resin; prewashed with a 10% hydrochloric acid solution.
- 4.8. BRIJ-35 detergent concentrate, Technicon Chemical Formula No. AR110-62.

#### AAI SYSTEM (DRINKING WATER)

##### 4.9. Hydrochloric Acid (1N)

Slowly add 83 ml of concentrated hydrochloric acid to approximately 500 ml of distilled water and dilute to 1 liter.

##### 4.10. Barium Chloride Stock Solution

Dissolve 1.526 g of barium chloride dihydrate in distilled water. Add 160 ml 1N hydrochloric acid and dilute to 1 liter.

##### 4.11. Working Color Solution

Dissolve 0.1182 g of methylthymol blue pentasodium salt in approximately 100 ml of distilled water. After the methylthymol blue dissolves, add 25 ml of barium chloride stock solution using a volumetric pipette and 450 ml of 95% ethanol and dilute to 1 liter with distilled water.

#### AAII System (River and Lake Samples, Sediment and Hi-Vol Extracts and Sulphation Rate Samples)

##### 4.12. Hydrochloric Acid (1.6N)

Slowly add 133 ml concentrated hydrochloric acid to approximately 500 ml water and make up to 1000 ml.

##### 4.13. Barium Chloride Stock Solution

Dissolve 1.9075 g barium chloride dihydrate in water and make up to 1 liter with distilled water. (This assumes a methylthymol blue purity of 100%.)

**NOTE:** For each new lot number of methylthymol blue, the purity must be determined (see 4.15) and the weight of barium chloride dihydrate must be adjusted according to the following:

$$(\% \text{ Purity} \times 1.9075 \text{ g}) / 100 = \text{new weight (g)}$$



#### 4.14. Methylthymol Blue Stock Solution

Dissolve 2.364 g methylthymol blue pentasodium salt in 800 ml distilled water. Add 50 ml 1.6 N hydrochloric acid and dilute to 1 liter.

#### 4.15. Methylthymol Blue Purity Check

Dilute 5 ml methylthymol blue stock solution to 273 ml with distilled water in a graduated cylinder. Read the absorbance, A, of this solution at 435 nm using a 1 cm cell. Calculate the purity according to the following:

$$\% \text{ Purity} = 105.82A$$

Record methylthymol blue lot number, % purity, weight of barium chloride dihydrate calculated in 4.13 and date of test.

#### 4.16. Methylthymol Blue Working Solution

To a 1 liter volumetric flask containing 800 ml ethanol add 50 ml methylthymol blue stock solution and 20 ml barium chloride stock solution. Dilute to 1 liter with distilled water.

#### 4.17. Sodium Hydroxide (0.18N)

Dissolved 7.2 g of sodium hydroxide and 10 drops of BRIJ-35 in 500 ml of distilled water and dilute to 1 liter.

#### ALL SAMPLES

#### 4.18. Sulphate Stock Solution (1000 mg/l $\text{SO}_4$ )

Dissolve 1.47882 g anhydrous sodium sulphate in distilled water and dilute to 1 liter.

#### 4.19. Sulphate Working Standards

AAI System (0 - 100 mg/l range): Dilute 0, 2, 5, 10, 25, 50, 75, 90, 100 and 120 ml aliquots of sulphate stock solution to 1 liter to give working standards with sulphate concentrations of 0, 2, 5, 10, 25, 50, 75, 90, 100 and 120 mg/l respectively.

AAII System (1 - 50 mg/l): Dilute 0, 5, 10, 15, 20, 25, 30, 40 and 50 ml stock solution to 1 liter. This provides working standards with sulphate concentrations of 0, 5, 10, 15, 20, 25, 30, 40 and 50 mg/l respectively.

#### 4.20. Quality Control Stock Solutions

AAI (1 - 100 mg/l): Dissolve  $1.71525 \pm 0.00005$  g sodium sulphate (dried for 1 hour at 150°C) to 1 liter with distilled water.

AAII (1 - 50 mg/l): Dissolve 2.9576 g sodium sulphate (dried as above) to 1 liter with distilled water.

#### 4.21. Quality Control Working Solutions

AAI (1 - 100 mg/l): Dilute 50 ml and 10 ml of appropriate quality control stock solution to 1 liter with distilled water to give QC-A and QC-B solutions with sulphate concentrations of 58 and 11.6 mg/l respectively.

AAII (1 - 50 mg/l): Dilute 50 ml and 10 ml of appropriate quality control stock solution to 4 liters to give QC-A and QC-B solutions with sulphate concentrations of 25 and 5 mg/l respectively.

#### 4.22. Dilution Water

Prepare dilution water containing 5 mg/l sulphate (AAII only).

#### 4.23. Daily Sensitivity Checks

AAI (1 - 100 mg/l): Prepare solutions that will provide 100% (High) and 10% (Low) of full scale response to monitor within-run sensitivity changes.

AAII (1 - 50 mg/l): Similarly, prepare solutions that will provide 80% (High) and 20% (Low) of full scale response.

#### 4.24. Ion Exchange Column

Place 20 ml exchange resin in a 250 ml beaker and wash free of fine particulates with water. Convert to  $H^+$  form by washing twice with 50 ml volumes of dilute hydrochloric acid and wash with water until washings are neutral (test with litmus paper). Store under water for future use. Add converted resin to ion exchange column keeping resin covered with water at all times. This prevents a decrease in ion exchange capacity caused by the presence of air bubbles. Plug ends with glass wool.

#### Soil and Sediments Only

4.25. Calcium chloride ( $CaCl_2 \cdot 2H_2O$ ) reagent grade powder.

#### 4.26. Calcium Chloride (0.01 M)

Dissolve 2.94 g calcium chloride in distilled water and dilute to 2 liters.

### 5. Procedure

Aqueous samples may be analyzed directly by 5.4. Preliminary extractions are not required.

#### 5.1. Soil and Sediment Extracts

- 5.1.1. Weigh 25 g air dried soil in a flask and add 50 ml 0.01 M calcium chloride. Place on shaker.
- 5.1.2. Shake vigorously for 30 minutes.
- 5.1.3. Filter through Whatman No. 30 and collect filtrate.
- 5.1.4. Determine sulphate on filtrates as described in 5.4.
- 5.1.5. Report extractant used when reporting results.

#### 5.2. Hi-Vol Filter Extraction

- 5.2.1. Cut a strip 19 mm x 254 mm from the central portion of a folded sample filter.
- 5.2.2. Cut strip into small portions and place in a 100 ml beaker with 30 ml water.

- 5.2.3. Cover beaker and place on hot plate at 80°C. Heat for approximately 1.5 hours, replenishing any water loss as required.
- 5.2.4. Cool and pour beaker contents through filter paper into a 100 ml graduated cylinder.
- 5.2.5. Add 10 ml water and reheat for a further 5 - 10 minutes. Cool contents and pour through same filter. Wash any residue from beaker into filter with small quantities of water.
- 5.2.6. Make extract volume to 50 ml with water, mix and transfer portion to test tube. Analyze according to 5.4. (These extracts are currently run on an AAI system. Greater dilution than described here is usually necessary since the system is described for the analysis of river and lake samples.)

### 5.3. Dustfall Preparation

- 5.3.1. Dustfall fractions are determined as in The Determination of Dustfall, however a representative aliquot of the filtrate is taken and analyzed for sulphate. Sulphate is therefore determined on the soluble fraction only.

### 5.4. Sulphate Determination

- 5.4.1. Rinse a culture tube with approximately 10 to 15 ml of well shaken sample. Discard, and then fill the rinsed culture tube with sample.
- 5.4.2. Place culture tube containing the sample into a test tube rack in such a position that it may easily be correlated with the sample number on the bench sheet. Write the appropriate sample number of the first and last sample tube in each row.
- 5.4.3. Set up the AutoAnalyzer according to Figure 1 or Figure 2. Cleaning, set-up and checking procedures are described in the user's manual for each manifold. The AAI system is currently used for the 1 - 100 mg  $\text{SO}_4/\text{l}$  measurement of drinking water, sewage, industrial waste and sediment extracts. The AAI system is currently employed for the 1 - 50 mg  $\text{SO}_4/\text{l}$  range for river and lake samples and Hi-Vol extracts. Greater dilutions than shown in Figures 1 and 2 may be required for sediment and Hi-Vol extracts, sewage and industrial waste samples.
- 5.4.4. **Sampler Loading Sequences**
  - 5.4.4.1. Each run of samples will include all of the following:
    - Set of standards (STDS)
    - Distilled water blank (BI)
    - Quality control samples (A, B)
    - Sensitivity monitoring standards, high and low (H, L)
    - Samples in groups of 10 or less (10).

5.4.4.2. The loading sequence for Technicon AAI system is:  
STDS; 2 Bl; A; B; n(10; L; H; Bl)  
where n is the number of repetitive units of samples.

5.4.4.3. The loading sequence for Technicon AAI System is:  
H; Bl; 2H; STDS; Bl; A; B; Bl; n(10; Bl; 10; L; H; Bl)  
where n is the number of repetitive units of samples.

**NOTE:** When loading the samples into the AutoAnalyzer sampler module, ensure that bench sheets are retained in the proper order by numbering and clipping them in order, one at a time, on a clipboard as each row of samples is loaded.

5.4.5. Commence run when there are sufficient samples to allow uninterrupted operation of the AutoAnalyzer.

5.4.6. Plot calibration curve for the series of standards. If the blanks and quality control checks do not agree with previous results, determine problem and correct before continuing.

5.4.7. Record the measured value of the QC-A, QC-B and the STD. CAL (the latter for AAI system only) setting during the run. Record the value of the Highs and Lows and if necessary, apply a within run sensitivity correction.

5.4.8. Read each sample peak using the calibration chart and record the result.

## 6. Calculation and Reporting

Multiply the reading by the dilution factor (DF):

$$DF = \frac{\text{diluted volume}}{\text{aliquot volume}}$$

For both systems and operational ranges, report results to 3 significant figures.

## 7. Precision and Accuracy

For river and lake samples run on the 1 - 50 mg/l range (AAI system) the standard deviations of duplicate samples are 0.420 for 0 - 20% of the range, 0.536 for 20 - 50% of the range and 0.673 for 50 - 100% of the range. Standard deviations of duplicate samples run on the 1 - 100 mg/l range (AAI system) are 0.54 for 0 - 20% of the range, 0.59 for 20 - 50% of the range and 0.57 for 50 - 100% of the range.

For the AAI system (0 - 100 mg/l range) calibration is controlled by 2 independently prepared long term standards (QC-A and QC-B) with sulphate levels of 58 mg/l and 11.6 mg/l, in such a way that (A + B) and (A - B) do not vary by more than 3.0 mg/l (3 standard deviations) from their long-term means. For the AAI system (0 - 50 mg/l range) accuracy is controlled similarly with QC-A = 25 mg/l, QC-B = 5 mg/l and control limits of 1.43 mg/l.

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## SULPHATE

### Automated Ion Chromatography Method B

#### SUMMARY

<b>Matrix.</b>	This method is routinely used for sulphate determinations on precipitation samples, some surface waters, and some air particulate samples.
<b>Substance determined.</b>	Sulphate ion, $\text{SO}_4^{=}$ .
<b>Interpretation of results.</b>	Results are reported as mg/l sulphate.
<b>Principle of method.</b>	Via ion chromatography (HPLC), sulphate is separated from other anions in samples using a column packed with ion exchange resin and an eluent composed of a mixture of sodium bicarbonate and sodium carbonate. Sulphate is converted to the acid form by ion exchange and its concentration is determined from the conductivity of the sulphuric acid produced. Manually drawn calibration curves are used.
<b>Time required for analysis.</b>	Time required for analysis depends upon eluent composition concentration and flow rate as well as the size of the separator column. With the experimental conditions specified for this procedure about 8 min per sample are required.
<b>Range of application.</b>	a) 0.05 - 5.00 mg/l. b) 0.05 - 10.0 mg/l.
<b>Standard deviation.</b>	Between run standard deviations are 0.034 in the 0.06 - 2.0 mg/l range; 0.078 in the 2.0 - 5.0 mg/l range and 0.167 in the 5.0 -10.0 mg/l range based on duplicate samples.
<b>Accuracy.</b>	Control is maintained by 2 independently prepared quality control standards (QC-A and QC-B) at 80 and 20% of range such that (A + B) and (A - B) do not vary by more than 0.14 mg/l (3 standard deviations) from their long-term means.
<b>Detection criteria.</b>	0.056 mg/l.
<b>Interferences and shortcomings.</b>	Oxalate at concentrations of 50 mg/l can interfere with the measurement of sulphate.

**Minimum volume  
of sample.**

15 ml if procedure is automated as described here.

**Preservation and  
sample container.**

Polyethylene bottles are suitable for sample storage. No preservative is necessary. Samples are refrigerated.

**Safety  
considerations.**

Caution should be exercised when handling sodium hydroxide, concentrated sulphuric acid and concentrated hydrochloric acid. Pressure developed in the IC system should not exceed 500 - 600 psi. Machine should be operated with door closed in case columns shatter.

## SULPHATE

### Automated Ion Chromatography Method B

#### 1. Introduction

Sulphate is the major anion in precipitation samples and consequently, this method was designed primarily for sulphate determination; however, it is also routinely used for the simultaneous measurement of chloride and nitrate.

Using a modified Dionex ion chromatograph system 10, anions are separated in a separator column by reaction with an ion exchange resin and an eluent. The anions are then converted to their acid form in a suppressor column (e.g. sulphate to sulphuric acid). The sodium carbonate-bicarbonate eluent becomes carbonic acid. A conductivity meter measures the conductivity of each anionic species against the carbonic acid eluent background. A precolumn is used to trap foreign matter thereby extending the life of the separator column. In order to accommodate automation, modifications are made allowing automatic sample injection.

#### 2. Interferences and Shortcomings

Each anionic species on the ion chromatograph is identified by its retention time: the time between sample injection and optimum peak height development. If the retention times of two anions are similar and one ion is present in considerably higher concentrations than the other, the broader and higher peak formed by this high concentration will overlap the peak of the lesser ion. Consequently, the capacity to determine an anion at low concentrations depends upon the concentrations of other ions having similar retention times. Oxalate peaks overlap sulphate peaks at oxalate concentrations of 50 mg/l.

Interferences may also be caused by the presence of ions which form strong complexes with the anions measured, or cause the anions to precipitate when mixed with the alkaline buffer. The latter is unlikely due to the low ionic strength of precipitation samples.

#### 3. Apparatus

##### 3.1. Dionex Ion Chromatographic System 10 including following:

- 3.1.1. anion precolumn, 3 x 150 mm; anion separator column (analytical), 3 x 250mm; anion suppressor column, 6 x 250 mm.
- 3.1.2. sample loop, 0.30 ml volume, tubing coiled.
- 3.1.3. #1 pump - eluent delivery at 40% flow.  
#2 pump - regenerant delivery at 60% flow.
- 3.1.4. regeneration time (normal cycle: 10 min. regeneration followed by 30 min. rinse with distilled, deionized water).



- 3.1.5. power - 115 volt AC, 60 HZ, 20 amp power source.
- 3.1.6. compressed air cylinder operated at 75 - 85 psi to activate valves.
- 3.1.7. chart recorder - 2 pen - must accept 1 V signal or less.
- 3.1.8. solenoids for sample injector valve.
- 3.1.9. 4-way sliders for sample valve.
- 3.2. Signal relay box - designed to handle 3 chromatographic channels.
- 3.3. Supergrator 3 (Columbia Scientific Industries).
- 3.4. AutoAnalyzer System including the following:
  - 3.4.1. large industrial model sampler - modified so that sampling events are controlled by Supergrator III program.
  - 3.4.2. AAI proportioning pump.
  - 3.4.3. glassware and pump tubing as in Figure 3.
  - 3.4.4. transmission tubing
- 3.5. Pipettes, 5, 10, 15, 20, 30, 40, 50 100 ml, volumetric.
- 3.6. Reagent bottles, 1000 ml to 2000 ml capacity.
- 3.7. Volumetric flasks, 1 liter, 2 liter capacity.
- 3.8. Weighing boats, glass "funnel" type, powder funnel.

#### 4. Reagents

- 4.1. Sodium chloride ( $\text{NaCl}$ ), reagent grade, anhydrous.
- 4.2. Sodium sulphate ( $\text{Na}_2\text{SO}_4$ ), reagent grade, anhydrous.
- 4.3. Potassium nitrate ( $\text{KNO}_3$ ), reagent grade, anhydrous.
- 4.4. Potassium chloride ( $\text{KCl}$ ), reagent grade, anhydrous.
- 4.5. Sodium carbonate ( $\text{Na}_2\text{CO}_3$ ), reagent grade, anhydrous.
- 4.6. Sodium bicarbonate ( $\text{NaHCO}_3$ ), reagent grade, anhydrous.
- 4.7. Sodium hydroxide ( $\text{NaOH}$ ), reagent grade, anhydrous.
- 4.8. Sulphuric acid ( $\text{H}_2\text{SO}_4$ ), reagent grade, concentrated.
- 4.9. Hydrochloric acid ( $\text{HCl}$ ) reagent grade, concentrated.
- 4.10. Distilled, deionized water.

**NOTE:** All reagents and standards are prepared with distilled, deionized water and volumetric, class A pipettes and flasks. All glassware must be washed with 4% HCl and thoroughly rinsed with distilled, then distilled, deionized water, before use.

**4.11. Super Stock Standard Solutions (300 mg/l Cl; 400 mg/l NO<sub>3</sub> as N; 2000 mg/l SO<sub>4</sub>)**

This is a combined stock solution prepared by weighing 0.4946 g sodium chloride, 2.8872 g potassium nitrate and 2.9572 g sodium sulphate on an analytical balance weighing to 4 decimal places. Combine reagents in a volumetric flask and make to 1 liter with distilled, deionized water.

**4.12. Intermediate Stock Standard Solutions (30 mg/l Cl; 40 mg/l NO<sub>3</sub> as N and 200 mg/l SO<sub>4</sub>)**

Pipette 100 ml super stock standard solution and dilute to 1 liter in a volumetric flask.

**4.13. Working Standards**

Working standards are prepared according to the following table, using distilled, deionized water.

% Range	Conc. mg/l			Volume Intermediate
	SO <sub>4</sub>	Cl	NO <sub>3</sub> -N	Stock Solution
10	1.00	0.15	0.20	5 ml/l
20	2.00	0.30	0.40	10 ml/l
40	4.00	0.60	0.80	20 ml/l *
60	6.00	0.90	1.20	30 ml/l
80	8.00	1.20	1.60	40 ml/l
100	10.00	1.50	2.00	50 ml/l

\* The 40% standard is also used as an in-run sensitivity check and is therefore prepared in 2 liter volumes (40 ml/2 l).

**4.14. Quality Control Super Stock Solution**

This is prepared exactly as for the calibration super stock standard solution (reagent 4.11); however, a different supply of chemicals is used.

**4.15. Quality Control Intermediate Stock Solution**

Pipette 100 ml quality control super stock solution into a 1 liter volumetric flask and dilute to the mark.

**4.16. Quality Control Working Standards**

An 80% (QC-A) and 20% (QC-B) standard are prepared by diluting 80 ml and 20 ml of quality control intermediate stock solution respectively, each to 2 liters. New standards should be run simultaneously with old standards at least once.

**4.17. Eluent Stock Solution**

Combine 25 g sodium carbonate and 25 g sodium bicarbonate in a 1 liter volumetric flask and dilute to the mark.

**4.18. Working Eluent Solution (0.003M NaHCO<sub>3</sub>/0.0024M Na<sub>2</sub>CO<sub>3</sub>)**

Pipette 20 ml eluent stock solution into a 2 liter volumetric flask and dilute to the mark.

**4.19. Spike Solution**

Combine 5 g sodium carbonate and 5 g sodium bicarbonate in a 1 liter volumetric flask and dilute to the mark.

**NOTE:** This is an estimate, which is easy to work with, yet effectively eliminates the negative dip due to loss of the baseline without adding a carbonate peak to the scan from the automated system (8.3).

**4.20. Regeneration Solution (1N H<sub>2</sub>SO<sub>4</sub>)**

Dilute 112 ml concentrated sulphuric acid to 4 liters with distilled, deionized water.

**4.21. Sodium Hydroxide Scrubber Solution (6.2N)**

Dissolve 250 g sodium hydroxide in distilled, deionized water and dilute to 1 liter.

**NOTE:** This reagent is used as a gas scrubber solution to provide carbon dioxide free air to the manifold air supply and maintain a carbon dioxide free atmosphere over the spike solution.

**5. Procedure**

REFER TO INSTRUCTION MANUAL'S "INSTALLATION" GUIDE FOR SET UP PROCEDURE. AIR SUPPLY AND RECORDER ARE ALSO REQUIRED.

**5.1. Operating Conditions:**

Cycle: analytical mode = 9 - 10 hrs; regeneration mode = 0.5 - 1 hr.

Eluent: composition = 0.003M NaHCO<sub>3</sub> and 0.0024M Na<sub>2</sub>CO<sub>3</sub>  
flow rate  $\approx$  180 ml/hr (40% flow)

Sample: automated pretreatment = An AAI pump is used to draw sample and mix it with spike solution ((0.060M in NaHCO<sub>3</sub> and 0.048M in NaHCO<sub>3</sub>) in v/v ratio 2.00:0.10 then resample and pump the spiked sample to the sample loop via the injection valve. Sample volume = 0.30 ml.

Precolumn: size = 3 mm I.D. x 125 mm

Separator column: size = 3 mm I.D., length = 250 mm

Suppressor column: size = 6 mm I.D. x 250 mm. regenerant = 1N H<sub>2</sub>SO<sub>4</sub>  
regeneration cycle: 10 min acid 60% flow  $\approx$  275 ml/hr  
30 min H<sub>2</sub>O

Detector: conductivity meter, 10  $\mu$ S/cm full scale

Chart recorder: Pen 1 = high analytical range, full scale equivalent to 10  $\mu$ S/cm.

Pen 2 = low analytical range, full scale equivalent to 5  $\mu$ S/cm.

Calibration: Blank plus 6 mixed standards, 10, 20, 40, 60, 80, 100% full scale. Calibration curve based on peak height.

Calibration range: Pen 1 (1V) =  $\text{SO}_4$ : 0.05 - 10.00 mg/l;  $\text{NO}_3\text{-N}$ : 0.01 - 2.00 mg/l; Cl: 0.01 - 1.50 mg/l.

Pen 2 (0.5V) =  $\text{SO}_4$ : 0.05 - 5.00 mg/l;  $\text{NO}_3\text{-N}$ : 0.01 - 1.00 mg/l; Cl: 0.01 - 1.20 mg/l.

## 5.2. Machine Set-up

- 5.2.1. With power and air supply conditions set (see Dionex Operation Manual) and appropriate reagents in reservoirs, select ELUENT (.004M  $\text{NaHCO}_3$ /.0024  $\text{Na}_2\text{CO}_3$ ) and allow to run through suppressor column at 80% flow ( $\approx$ 370 ml/hr) for 15 - 30 min, to stabilize column.

**NOTE:** It is assumed that suppressor column is freshly regenerated at beginning of run (Section 5.5) and that all reagent lines are air free (i.e.: pump has been primed for all reagents - see Dionex Manual for procedure) ensuring that no air is introduced into the system.

- 5.2.2. While stabilizing column, set up recorder using the Dionex Ion Chromatograph conductivity meter by setting meter to zero mode and with both pens of recorder at 1V, zero the pens.

- 5.2.3. Set mode switch to CAL and adjust both pens to full scale (i.e. 100 chart lines) using SPAN adjustment on underside of recorder.

**NOTE:** ZERO mode should give zero signal on conductivity board of Dionex. CAL mode should give 1 V signal (i.e. full scale deflection on conductivity board of Dionex). If these conditions are otherwise consult Dionex manual for adjustments (8.2).

- 5.2.4. Recheck ZERO and CAL, then select LIN mode and 10  $\mu$ mho scale. Set pump flow to 40% ( $\approx$ 180 ml/hr) and put all columns on line. Offset conductance to zero and bring each pen up to 5 chart lines with the zero adjust on the recorder to accomodate any drift in signal. Change pen 2 to .5V.

**NOTE:** If flow is higher, the pressure will be greater than 500 psi and may cause columns to explode, or leak.

- 5.2.5. Turn on pump with sample probe in wash.

- 5.2.6. Program the Supergrator with suitable program: PGM 1 for the 500 mm column and PGM 2 for the 250 mm column.

**NOTE:** Refer to Supergrator 3 Operating Manual (8.4) for program explanation.

As long as the instrument is not switched off, the program is retained in the memory and may be called up each day or edited as desired.

- 5.2.7. The system set up should correspond to Figure 3.

5.2.8. Turn on sampler and relay box.

### 5.3. Sample Run

5.3.1. Set up samples in order for day's run as follows:

10%, 20%, 40%, 60%, 80%, 100% STDS, QC-A(20), QC-B(80); 5 samples, 40% in-run std, 5 samples, QC-A, 40% in-run STD. One sample should be a duplicate from previous day's run.

5.3.2. Set Supergrator to NORMAL mode and start run.

### 5.4. Clean-up and Shut-down

5.4.1. Turn off separator column. Select DI water and allow it to rinse suppressor column and conductivity cell until the low level registers on the conductivity meter.

5.4.2. If necessary (light yellow color) regenerate the suppressor column (see section 5.5). During this phase of operation, pump 1 can be turned off.

5.4.3. Turn off air and power on instrument first, then turn air off at the cylinder valve.

5.4.4. Maintenance of instrument is described in the Dionex manual and should be followed and recorded in the log book (inside top door of Dionex).

### 5.5. Suppressor Column Regeneration

5.5.1. Suppressor column should be set on BYPASS/REGEN and should have been flushed with DI water after a run in which the column was used.

**NOTE:** To flush with DI water, select water, turn suppressor column toggle up and flush for about 5 minutes.

5.5.2. With timer set at 10 min for 1N sulphuric acid regenerant and 30 min for water rinse, push in the START button. This starts the regeneration (back) pump going. Pump is set at 60% flow ( $\approx 275$  ml/hr). At the end of the cycle (40 min) the pump will shut off automatically.

**NOTE:** Suppressor column is usually regenerated once a day at the end of each day.

## 6. Calculation and Reporting

Peak heights are measured and for all standards on 1V and .5V pens (100%, 80, 60, 40, 20, 10) and are plotted on one graph (for convenience) as peak height (mm) vs conc (%).

QC-A and QC-B are read off graph (as % concentration, and then converted to mg/l) and checked against control limits.

Peak heights of samples are read as % concentration, converted to mg/l, with a correction for sensitivity changes during the run.

## 7. Precision and Accuracy

Based on duplicate samples, standard deviations for sulphate measurements are 0.034 in the 0.06 - 2.0 mg/l range; 0.078 in the 2.0 - 5.0 mg/l range and 0.167 in the 5.0 - 10.0 mg/l range.

This method is applicable to a variety of anions and the standard deviations are as follows:

Chloride	0.017	0.03 - 1.20 mg/l range
Nitrate	0.020	0.03 - 0.20 mg/l range
	0.012	0.20 - 0.50 mg/l range
	0.016	0.50 - 1.00 mg/l range

Accuracy is controlled by 2 independently prepared standards (QC-A and QC-B) at 80% and 20% of range (8.0 mg/l and 2.0 mg/l) such that (A + B) and (A - B) do not vary by more than 0.14 mg/l (3 standard deviations) from the long-term mean of (A - B).

## 8. Bibliography

- 8.1. Crowther, J. and McBride, J. (1978). Analysis of Precipitation Samples by Ion Chromatography. Part 1 - Determination of Anions. Ontario Ministry of the Environment, Laboratory Services Branch, Toronto, Ontario.
- 8.2. Dionex Ion Chromatographs 10, 14, 16. Operation and Maintenance. Dionex Corporation, Sunnyvale, California.
- 8.3. McBride, J., Clarke, K., Crowther, J., Lindow, O. and Rawlings, M.W., (1979). Automation of Anion Chromatographic System. Ontario Ministry of the Environment, Laboratory Services Branch, Toronto, Ontario.
- 8.4. Supergrator 3 Operating Manual ROM 326, Columbia Scientific Industries.







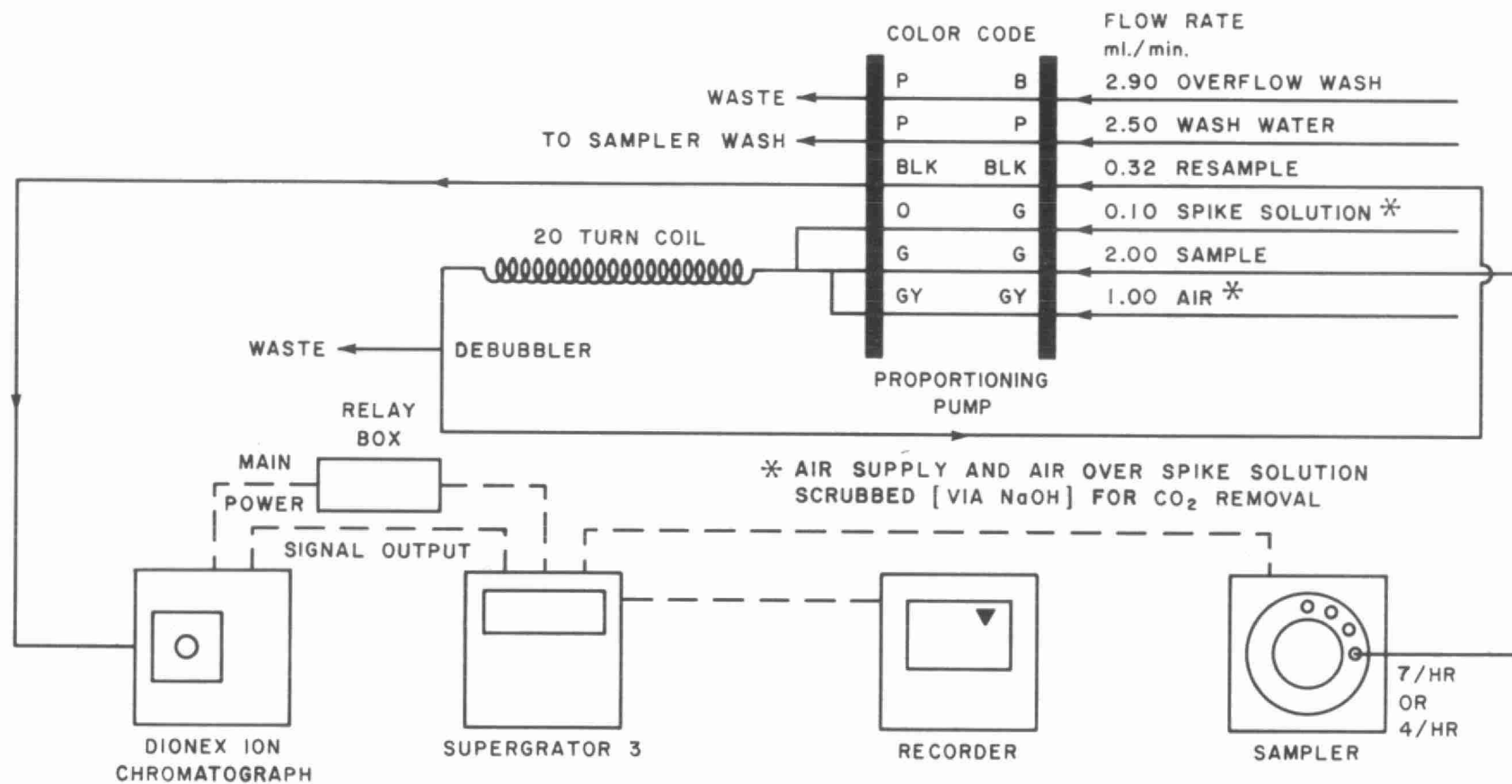


FIGURE 3 — AUTOMATED ION CHROMATOGRAPH SYSTEM (ANIONS)

## THE DETERMINATION OF SULPHIDE

Sulphide is formed by bacterial reduction of sulphate and organic sulphur compounds under anaerobic conditions. It is, therefore, commonly found in domestic wastewater, industrial wastewater, sludges, hypolimnions of stratified lakes and any other aquatic systems where anaerobic conditions prevail. As a result, concentrations in surface waters are negligible. Hydrogen sulphide may be found in various forms depending on the pH of the water. At pH 5, it is almost entirely found as  $H_2S$  while at pH 9, it is almost entirely  $HS^-$ . The  $S^{2-}$  anion is generally not found in natural waters. The characteristic hydrogen sulphide odour of "rotten eggs" is highly objectionable.

Sulphide is an important parameter in waste treatment monitoring. Oxidation of sulphide to sulphuric acid in concrete sewer pipes leads to "crown corrosion". Soluble sulphides in excess of 200 mg/l are toxic to bacteria and will inhibit sludge digestion. In order to protect aquatic life, concentrations of undissociated hydrogen sulphide should not exceed 0.002 mg/l.

Samples containing sulphides are unstable and results must be interpreted with caution.

### Sample Handling and Preservation

#### Water, Sewage

Samples must be collected with a minimum amount of agitation as sulphides are highly volatile and susceptible to oxidation by dissolved oxygen. The container should be filled completely so that no air bubbles remain after the sample is capped. Immediate delivery to the laboratory is essential. Where possible a field analysis should be performed.

Preservation is accomplished by adding 2 ml of a 2N zinc acetate solution to the sample followed by the dropwise addition of a 5% sodium carbonate solution until precipitation is complete. Consultation with laboratory staff is recommended when sulphide analyses are required.

#### Soil and Sediment

Soil samples are collected in pomade jars. When dried for analysis, the drying factor is determined.

### Selection of Method

Method A is an inert gas displacement ammonium molybdate method. Analysis is performed colorimetrically using a manual colorimeter. This is the method of choice for most sample types because of its simplicity.

Method B is a semi-quantitative Hach lead acetate spot test and is generally only used to obtain a rough estimate of the sulphide concentration. This method is most commonly used for domestic water, sewage and industrial waste samples. Method C is an ion specific electrode method used by the London regional laboratory for aqueous samples.

Method D is a methylene blue spectrophotometric method which is useful on relatively clean water samples.

## SULPHIDE

### Inert Gas Displacement - Ammonium Molybdate Method A

#### SUMMARY

<b>Matrix.</b>	Water, sediment, soil and sewage sludge samples can be analyzed by this method.
<b>Substance determined.</b>	Hydrogen sulphide.
<b>Interpretation of results.</b>	Results are reported as mg/l as H <sub>2</sub> S for liquid samples and µg/g for solid samples. Caution must be exercised in interpreting the data, as some organic sulphur compounds may release sulphide under the conditions of the test.
<b>Principle of method.</b>	Samples are treated with dilute hydrochloric acid to convert sulphides to hydrogen sulphide. This gas is then sparged from solution with nitrogen gas and bubbled through an acidified ammonium molybdate absorbing solution. The absorbance of the blue sulpho-molybdate complex is measured at 700 nm.
<b>Time required for analysis.</b>	Approximately 15 sulphide analyses may be performed per day.
<b>Range of application.</b>	The operating range is from 1 to 15 mg/l as hydrogen sulphide. Higher concentrations can be analyzed after sample dilution.
<b>Standard deviation.</b>	The standard deviation is 0.2 at 2.2 mg/l.
<b>Accuracy.</b>	Not established.
<b>Detection limit.</b>	.02 mg/l as H <sub>2</sub> S.
<b>Interferences and shortcomings.</b>	Acidification is only moderate and under the conditions of the test insoluble sulphides (e.g. lead and mercury) will not react. Some easily hydrolyzable organic sulphur compounds may decompose to yield hydrogen sulphide under the conditions of the test. Interference is reduced by isolating hydrogen sulphide from the sample.
<b>Minimum volume of sample.</b>	1000 ml for liquid samples. At least 1 gram of dried sample is required.

**Preservation and  
sample container.**

Glass bottles are recommended for aqueous samples. Preservation is accomplished by adding 2 ml of a 2N zinc acetate solution to the sample followed by the dropwise addition of a 5% sodium carbonate solution until precipitation is complete. Solid samples should be collected in glass jars. All samples should be refrigerated.

**Safety  
considerations.**

Hydrogen sulphide is an extremely toxic gas. Low concentrations in a laboratory environment may produce dizziness, headache and nausea.

Work should be carried out in a well ventilated fumehood.

## SULPHIDE

### Inert Gas Displacement - Ammonium Molybdate Method A

#### 1 Introduction

Samples of water, sediment, soil or sewage sludge, preferably as is, are treated with dilute hydrochloric acid to convert sulphides to hydrogen sulphide. This gas is then sparged from solution by a stream of nitrogen gas and bubbled through acidified ammonium molybdate absorbing solution. The absorbance of the blue sulpho-molybdate complex at 700 nm is proportional to the sulphide concentration.

This method is preferred for waste, waters, sediments, soils and sludges. Interferences are reduced by isolating hydrogen sulphide gas from the liquid.

#### 2. Interferences and Shortcomings

Since acidification is only moderate and of relatively short duration under the conditions of the test, insoluble sulphides (e.g. of lead and mercury) will not react. Some easily hydrolyzable organic sulphur compounds may decompose to yield hydrogen sulphide under the conditions of the test.

#### 3. Apparatus

- 3.1. Generator and absorption assembly. Assemble apparatus as shown in Figure 1. Epoxy cement is used for sealing Tygon tubes in culture tube caps. Cyclohexanone can be used for sealing Tygon tubing. Arrangement in use has 8 positions.
- 3.2. Culture tubes, Pyrex with screw cap, Corning or equivalent, 25 x 150 mm (generators).
- 3.3. Plastic tubing, Tygon Impinger nozzles of 1 cm length of Technicon 0.17 ml/min flow tubes inserted into transmission tubing 2 mm O.D.
- 3.4. Spectrophotometer cells, 1 cm path length matched and spectrophotometer, B & L Spectronic 20 or equivalent; 4 cm cells can be used with increase in sensitivity.
- 3.5. Boiling tubes, 50 ml, 2 cm I.D. graduated at 25 ml and 50 ml; 20 ml tubes.
- 3.6. Cylinder, nitrogen with pressure regulator (10 psig.).

#### 4. Reagents

- 4.1. Ammonium molybdate ( $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}$ ).
- 4.2. Hydrochloric acid (HCl), 12N, low sulphur content ACS grade.

- 4.3. Thioacetamide ( $\text{CH}_3\text{CSNH}_2$ ), crystals, reagent grade.
- 4.4. Zinc, activated with copper, reagent grade. Alternatively this can be prepared as follows:

Zinc, activated with copper. Mix 250 g zinc, 20 mesh, with 10 ml 10% copper sulphate and 250 ml distilled water. Drain off excess water and dry at  $105^\circ\text{C}$ . Sieve and discard fraction finer than 40 mesh. For convenience commercially available activated zinc can be used.

- 4.5. Sodium sulphide ( $\text{Na}_2\text{S} \cdot 9\text{H}_2\text{O}$ ), crystals, reagent grade.

- 4.6. Nitrogen - 99.97% purity.

- 4.7. **Ammonium Molybdate Reagent (0.25%).**

Dissolve 2.50 g ammonium molybdate in about 900 ml distilled water, adjust to pH 1.5 with concentrated HCl (about 4 ml) and dilute to 1 liter. Replace if any trace of turbidity or precipitation.

- 4.8. **Standard Thioacetamide Stock Solution (1000 mg/l  $\text{H}_2\text{S}$  equivalent).**

Dissolve 2.02050 g thioacetamide in water and dilute to 1 liter in a volumetric flask.

- 4.9. **Standard Thioacetamide Solution (10 mg/l  $\text{H}_2\text{S}$  equivalent).**

Dilute 10.00 ml stock solution to 1 liter with distilled water. Standardize 50.00 ml iodometrically with N/80 iodine and thiosulphate.

- 4.10. **Standard Thioacetamide Solution (1 mg/l  $\text{H}_2\text{S}$  equivalent).**

Dilute 100.0 ml of 10 mg/l thioacetamide solution to 1 liter with distilled water. Replace after one month or if deterioration is indicated by absorbance readings.

- 4.11. **Sodium Sulphide Stock Solution (approximately 1000 mg/l  $\text{H}_2\text{S}$ ).**

Dry crystals of sodium sulphide on filter paper. Dissolve about 0.75 g in boiled distilled water and make up to 100 ml (about 1000 mg/l). Standardize just before use by standard iodine-thiosulphate titration.

- 4.12. **Sulphide Standard Solution (approximately 10 and 1 mg/l).**

Prepare 10 mg/l and 1 mg/l standards by diluting appropriate aliquots of sulphide stock (4.11) with boiled distilled water. Prepare fresh solutions daily.

## 5. Procedure

- 5.1. Assemble the generators and absorber tubes required for two standards plus a blank, in addition to those required for samples. Check that the impinger tips are not plugged, and replace if questionable.

- 5.2. Check that there is an adequate supply of nitrogen in the cylinder. Open the main cylinder valve and adjust the pressure to 8 psig. Open the shut-off valve and adjust the control valve to give a slow rate of bubbling in the bubblers.
- 5.3. Pipette 1.00, 5.00 and 10.00 ml of 1 mg/l or 10 mg/l of thioacetamide standards into the generator tubes, depending on whether the samples are expected to contain up to 1.0  $\mu\text{g}$  or 100  $\mu\text{g}$   $\text{H}_2\text{S}$  in the aliquot used for the test; 20 ml of 10 mg/l for 200  $\mu\text{g}$   $\text{H}_2\text{S}$ . Two generator tubes should also be set up for blanks. The threaded portion of each generator tube should be wiped with stopcock grease to prevent leaks. In practice, once linearity has been established, the slope of the calibration curve can best be established by duplicate 10 ml standards. Check the thioacetamide bi-weekly against fresh sulphide standards in the same run.
- 5.4. Add approximately 1 g of copper activated zinc to each of the thioacetamide standards and to one of the blanks. Once the absence of blank readings for a lot of copper activated zinc has been established, the second blank can be discontinued.
- 5.5. Weigh out accurately, or pipette if liquid, a portion of each sample expected to contain up to 10, 50, 100 or 200  $\mu\text{g}$   $\text{H}_2\text{S}$  into the generator tubes. The maximum volume of liquid to be used is 40 ml. Liquid samples, if they contain particulate matter such as preserved samples, should be well shaken prior to taking the aliquot. Solid samples should be well blended.
- The drying factor should be determined separately on an original portion of sample.
- 5.6. Place the delivery tube from each generator into an appropriate volume of absorbing solution (either 4, 10, 25 or 50 ml). In any one run all absorbers should preferably contain the same volume of absorbant.
- The tip of the delivery tube must be located at the bottom of the absorber tube to permit efficient absorption.
- 5.7. Add 40 ml distilled water to each generator tube for solid samples and shake. For liquid samples, make up volume to 40 ml.
- 5.8. Check the inert gas flow; ensure that there is a slow rate of bubbling in all absorbers.
- It is necessary to provide positive gas pressure before placing the absorbers on line to prevent plugging of the fine orifices in the generator impingers.
- A low gas flow rate is required to minimize loss of  $\text{H}_2\text{S}$  at start-up, minimize foaming and particle entrainment which could plug orifices of impingers in absorbing solution. The efficiency of absorption is also reduced at high gas flows. Add anti-foam agent if there is a problem with foaming.
- 5.9. Add 4 ml HCl to each generator tube, screw down the cap of the tube immediately and rapidly to minimize initial loss of  $\text{H}_2\text{S}$ .
- 5.10. Continue until all generator tubes are in operation. Bubble nitrogen through tubes at a low flow rate for 45 - 60 minutes.
- Check to ensure that all absorbers are bubbling. Excessively low or no flow rate may indicate a gas leak or that an impinger has become plugged, in which case the test will have to be repeated.



5.11. At end of the gas displacement period (if there is any uncertainty) check for completion by placing tube in a second absorber tube with 1 ml absorbing solution. If no blue color develops in 15 minutes, the test can be considered complete. Remove the screw caps and impingers from the generator tubes before turning off the nitrogen to avoid plugging. Repeat the test with a larger aliquot (up to 40 ml) if the absorbance is close to the detection limit.

5.12. Zero the spectrophotometer on the blank absorbing solution at 700 nm and measure the absorbance of each solution. Prepare calibration curves for absorbance or determine the slope factor and determine  $\mu\text{g H}_2\text{S}$  in each portion of sample analyzed.

If desired a 4 cm cell can be used, such as in the Unicam SP 1800, to increase the sensitivity.

A tentative modified version of the method can be used for zinc acetate-sodium carbonate preserved water samples by selecting 1000 ml of sample to improve detection limits (0.002 mg/l  $\text{H}_2\text{S}$ ).

High absorbance readings indicate incomplete absorption of  $\text{H}_2\text{S}$ . Repeat the test with a smaller portion of sample, or if 4 ml absorbing solution has been used, with a larger volume of absorbing solution (25 ml or 50 ml).

If desired, a rough estimate of concentration can be obtained by diluting the colored solution with absorbing solution, measuring the absorbance and allowing for the dilution factor. The results will be low because of less efficient absorption at higher concentrations.

## 6. Calculation and Reporting

Results are reported as  $\mu\text{g/g H}_2\text{S}$  on an oven-dry weight basis for solid samples or as mg/l  $\text{H}_2\text{S}$  for liquid samples.

$$\mu\text{g/g H}_2\text{S} = \frac{\mu\text{g H}_2\text{S from calibration curve}}{\text{sample wt in grams} \times \text{drying factor}}$$

$$\text{mg/l H}_2\text{S} = \frac{\mu\text{g H}_2\text{S from calibration curve}}{\text{ml original sample}}$$

Concentration can also be determined using calculated slope factor.

## 7. Precision and Accuracy

Relative standard deviation above 10 per cent at the 0.2 mg/l  $\text{H}_2\text{S}$  level. The detection limit is 0.02 mg/l  $\text{H}_2\text{S}$  for a 40 ml aliquot.

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- 8.2. Donagi, A., Kendler, A. and Davidson, M. The direct ultra micro-determination of hydrogen sulphide in the atmosphere by the molybdenum blue method. Proc. Israel J. Chem. 5: 152, 1967.

## SULPHIDE

### Semi-Quantitative Hach Lead Acetate Test Method B

#### SUMMARY

<b>Matrix.</b>	This method is used for drinking water, sludges and sediment samples.
<b>Substance determined</b>	Hydrogen sulphide.
<b>Interpretation of results.</b>	Results are reported in mg/l as $H_2S$ as read from the Hach kit chart. Results are semi-quantitative.
<b>Principle of method.</b>	An Alka-Seltzer tablet is added to a 25 ml sample. The hydrogen sulphide released reacts with a lead acetate impregnated filter paper to produce lead sulphide. The color of the filter paper is then matched to the Hach color chart provided and the sulphide concentration determined.
<b>Time required for analysis.</b>	Each test requires approximately 5 minutes. The number of tests completed at one time is limited by the number of Hach tubes available.
<b>Range of application.</b>	0 - 5 mg/l. Samples in excess of 5 mg/l as $H_2S$ may be determined by dilution.
<b>Standard deviation.</b>	Variable depending on the uniformity of color on the filter and on variations between operator judgement.
<b>Accuracy.</b>	Semi-quantitative method.
<b>Detection limit.</b>	0.1 mg/l sulphide.
<b>Interferences and shortcomings.</b>	If effervescence reaches the filter paper, the slightly acidic nature of the solution causes some of the lead sulphide formed on the filter paper to redissolve.
<b>Minimum volume of sample.</b>	25 ml.
<b>Preservation and sample container.</b>	Glass or plastic bottles are suitable. Samples may be preserved by the addition of 2 ml 2N zinc acetate solution per liter of sample followed by dropwise addition of 5% sodium carbonate solution until precipitation is complete.

**Safety  
considerations.**

Hydrogen sulphide is an extremely toxic gas.  
Work should be carried out in a well ventilated fumehood.

## SULPHIDE

### Semi-Quantitative Hach Lead Acetate Test Method B

#### 1. Introduction

An Alka-Seltzer tablet is added to a slightly acidified sample in a plastic vial. Sulphide present in the sample is released as hydrogen sulphide gas. This gas discolors a disc of lead acetate impregnated paper placed in the cap of the vial by producing lead sulphide. The color of the paper is matched with a disc on the color chart provided and the sulphide concentration is determined.

#### 2. Interferences and Shortcomings

If effervescence reaches the lead acetate disc, the slightly acidic nature of the solution causes some of the lead sulphide formed on the disc to redissolve.

This method is semi-quantitative. For a more accurate measure of sulphide, an alternate technique must be used. The color is often not uniformly distributed over the disc and therefore matching of the color with the discs on the color chart provided is left to the judgement of the operator. Variation between the color matching of 2 different operators may be significant.

#### 3. Apparatus

3.1. Hach kit for the hydrogen sulphide test including the following:

- 3.1.1. lead acetate test paper
- 3.1.2. hydrogen sulphide color chart
- 3.1.3. plastic tubes with caps

#### 4. Reagents

- 4.1. Alka-Seltzer, registered trademark, Miles Laboratories, Elkhart, Indiana.
- 4.2. Hydrochloric acid, (HCl), concentrated, reagent grade.

#### 5. Procedure

- 5.1. Fill a plastic sample vial to the mark with the sample to be tested for hydrogen sulphide. For most accurate results, perform test on freshly collected sample. Aeration of the sample or allowing the sample to sit for a long period of time prior to analysis will result in loss of sulphide by aeration and oxidation.

- 5.2. Place a disc of hydrogen sulphide test paper inside the cap of the sample vial. Do not wet paper disc while handling it.
- 5.3. Add 1 - 2 drops concentrated hydrochloric acid to the sample.
- 5.4. Add an Alka-Seltzer tablet to the sample and IMMEDIATELY place the cap containing the test paper on the vial.
- 5.5. After the tablet has dissolved and effervescence has stopped, remove the test paper disc and compare the color with the color chart provided.

## **6. Calculation and Reporting**

Read results as given on the color chart and report.

## **7. Precision and Accuracy**

This test is semi-quantitative. Differences in operator judgement regarding color matching causes variations in reproducibility.

## SULPHIDE

### Specific Ion Electrode Method C

#### SUMMARY

<b>Matrix.</b>	This method is routinely used on all water samples.
<b>Substance determined.</b>	Sulphide ion.
<b>Interpretation of results.</b>	Results are reported in mg/l as H <sub>2</sub> S.
<b>Principle of method.</b>	When the electrode is placed in a solution containing sulphide ions, these ions migrate and set up a potential difference relative to the sulphide concentration. This potential is measured against a constant reference potential with a specific ion meter. Sulphide anti-oxidant buffer is added to all standards and samples.
<b>Time required for analysis.</b>	Approximately 20 samples can be analyzed per day.
<b>Range of application.</b>	0.01 to 100 mg/l as H <sub>2</sub> S.
<b>Standard deviation.</b>	Not established.
<b>Accuracy.</b>	Not established.
<b>Detection limit.</b>	0.01 mg/l.
<b>Interferences and shortcomings.</b>	Many interferences are overcome by placing the sulphide anti-oxidant buffer in the sampling bottle. Some organic sulphur compounds may decompose to yield hydrogen sulphide.
<b>Minimum volume of sample.</b>	50 ml.
<b>Preservation and sample container.</b>	Glass bottle with sulphide anti-oxidant buffer already added.
<b>Safety considerations.</b>	Hydrogen sulphide is an extremely toxic gas. Work should be carried out in a well ventilated fumehood.

## SULPHIDE

### Specific Ion Electrode Method C

#### 1. Introduction

The Ag/S electrode consists of a silver sulphide membrane bonded into an epoxy body. When the electrode is placed in a solution containing sulphide ions, sulphide ions migrate within the membrane setting up a potential difference proportional to the sulphide concentration. The potential is measured against a constant reference potential with a specific ion meter. Sulphide anti-oxidant buffer (SAOB) is added to all standards and samples to prevent oxidation as well as adjust the ionic strength and pH of the solution.

#### 2. Interferences and Shortcomings

Most interferences are overcome by adding the sulphide anti-oxidant buffer to the sample at the time of collection. Organic sulphur compounds may decompose to yield hydrogen sulphide.

#### 3. Apparatus

- 3.1. Volumetric flask.
- 3.2. Pipettes.
- 3.3. Folin digestion tubes; 50 ml.
- 3.4. Beakers, 100 ml
- 3.5. Magnetic stirrer
- 3.6. Sulphide ion specific electrode and Orion Model 901 Ionalyzer.

#### 4. Reagents

- 4.1. Ascorbic acid ( $C_6H_8O_6$ ), crystals, reagent grade.
- 4.2. Di-sodium EDTA ( $CH_2N(CH_2COOH)CH_2.COONa)_2.2H_2O$ ), crystals, reagent grade.
- 4.3. Sodium hydroxide, (NaOH) pellets.
- 4.4. Sodium sulphide nonahydrate ( $Na_2S.9H_2O$ ), crystals, reagent grade.
- 4.5. Nitrogen gas.
- 4.6. Potassium Nitrate ( $KNO_3$ ), pellets, reagent grade.

#### 4.7. Lead Perchlorate (0.1M)

Order from Orion cat. No. 94-82-06.

#### 4.8. Electrode Filling Solutions

4.8.1. Inner filling solution - order from Orion cat. No. 90-00-02.

4.8.2. Outer filling solution - 10% Potassium Nitrate ( $\text{KNO}_3$ ) solution adjusted to a pH of 13.5 (to match the SAOB solution) with NaOH.

#### 4.9. Deaerated Deionized Water

Purge a 2 liter glass bottle of deionized water with nitrogen gas ( $\text{N}_2$ ) for at least 15 minutes.

#### 4.10. Sulphide Anti-Oxidant Buffer (SAOB)

Add 35 g ascorbic acid, 67 g disodium EDTA, 500 ml deionized water, and with stirring, 200 ml of 10N sodium hydroxide (NaOH) or 80 g sodium hydroxide (NaOH) pellets to a 1 liter volumetric flask. Cool the mixture and dilute to 1 liter with deionized water.

**NOTE:** VERY CAUSTIC SOLUTION - pH GREATER THAN 13 WEAR EYE PROTECTION, GLOVES.

**NOTES:** Discard when the solution turns brown.  
Store in a refrigerator at  $4^\circ\text{C}$  until required.  
Put stopcock grease on the stopper of the volumetric flask.

#### 4.11. Stock Sulphide Solution (approximately 1330 mg/l)

Dissolve 10 g sodium sulphide ( $\text{Na}_2\text{S} \cdot 9\text{H}_2\text{O}$ ) in deaerated deionized water and dilute to 1 liter. Standardize the solution before each set-up. Store in refrigerator at  $4^\circ\text{C}$ .

**NOTE:** Sodium sulphide crystals deteriorate quickly when exposed to air.

**NOTES:** WEAR GLOVES AND EYE PROTECTION. USE WITH ADEQUATE VENTILATION. AVOID BREATHING VAPORS. AVOID CONTACT WITH ACIDS.

#### 4.12. Intermediate Stock Sulphide Solution

Dilute 10 ml of the stock sulphide solution to 1 liter with deionized water in a volumetric flask.

#### 4.13. Working Solutions

4.13.1 Dilute 100 ml of the intermediate stock solution to 1 liter with deionized water (1.33 mg/l) in a volumetric flask.

4.13.2. Dilute 10 ml of the intermediate stock solution to 1 liter with deionized water (.133 mg/l) in a volumetric flask.

### 5. Procedure

#### Calibration of Stock Sulphide Solution

5.1 Pipette 25 ml of the SAOB solution into a Folin tube and add 30 ml of the stock solution.



- 5.2. Empty this tube into a 100 ml beaker containing a stirring bar, and place on a magnetic stirrer.
- 5.3. Add lead perchlorate from a burette in 0.5 - 1.0 ml increments.
- 5.4. Record the titration volume and millivolt reading after each titrant addition.
- 5.5. When the millivolt change per increment begins to increase rapidly, reduce the increments to 0.1 ml.
- 5.6. Add lead perchlorate in 0.1 ml increments until a sharp decrease in millivolt reading occurs. Continue addition until a steady millivolt reading is achieved.
- 5.7. Plot millivolt readings vs ml of titrant on graph paper to determine the endpoint (inflection point).
- 5.8. Determine the concentration C, of the stock sulphide solution:

$$C = \frac{V_t}{V_s} \times 3200$$

Where:

V<sub>t</sub> = volume of titrant at the endpoint (ml)

V<sub>s</sub> = initial volume of stock standard (30 ml)

#### 5.9. Reference Electrode Preparation

##### 5.9.1. Double junction electrode model 90-02-00

- 5.9.1.1 Filling solutions must be replaced before each set-up.
- 5.9.1.2. Maintain the outer chamber level 1 inch above the sample level.
- 5.9.1.3. Maintain the inner chamber level 1 inch above the outer-chamber level.
- 5.9.1.4. Avoid touching the bottom of the electrode.

##### 5.9.2. Filling the inner chamber:

**NOTE:** WEAR GLOVES AND EYE PROTECTION

- 5.9.2.1. Unscrew the cap on the electrode and remove the spring and silver contact.
- 5.9.2.2. Flush the old solution out the vent hole and replace with fresh solution.
- 5.9.2.3. Rinse the spring and silver contact with deionized water and replace. Screw the cap on finger tight.

##### 5.9.3. Filling the outer chamber:

**NOTE:** WEAR GLOVES AND EYE PROTECTION

- 5.9.3.1. Use tissue to avoid fingerprints on the electrode (Methanol or Ethanol can be used to clean off fingerprints).
- 5.9.3.2. Invert the electrode to moisten the green O-ring inside the outer chamber.

- 5.9.3.3. Hold the electrode by the cap and push the outer sleeve up into the cap - draining the old filling solution.
- 5.9.3.4. Refill the outer chamber with the Potassium Nitrate ( $\text{KNO}_3$ ) solution, then repeat 5.9.3.3 and refill again.
- 5.9.3.5. Store electrodes in deionized water until ready to use.

#### 5.10. Sample Analysis

FOR INSTRUMENT CALIBRATION PROCEDURES REFER TO MANUFACTURER'S MANUAL.

**NOTE:** THE SOLUTIONS BEING ANALYZED ARE VERY CAUSTIC -HANDLE WITH CARE.

**NOTE:** Do not allow the magnetic stirring bar to hit the electrodes.

**NOTE:** Samples and standard solutions must be at the same temperature (usually room temperature). A  $1^\circ\text{C}$  temperature difference is equal to a 4% error in measurement.

- 5.10.1. Pour the contents of the sulphide sample tube into a 100 ml beaker and begin stirring.
- 5.10.2. With the instrument set on the CONCN. mode, place the electrodes in the beaker and allow the reading to stabilize.
- 5.10.3. Record the sample concentration directly from the instrument display.
- 5.10.4. Rinse the electrodes and pat dry before analyzing the next sample.
- 5.10.5. Re-calibrate the instrument every 3 hours.
- 5.10.6. When finished, store the electrodes in deionized water.

#### 6. Calculation and Reporting

Read hydrogen sulphide concentration directly from the instrument display.

Report results to 0.01 mg/l as  $\text{H}_2\text{S}$ .

#### 7. Precision and Accuracy

Not yet established.

## SULPHIDE

### Methylene Blue Spectrophotometric Method D

#### SUMMARY

<b>Matrix.</b>	This method is used routinely on water samples.
<b>Substance determined.</b>	Sulphide ion, $S^{2-}$ .
<b>Interpretation of results.</b>	Results are reported in mg/l as $H_2S$ . Caution must be exercised when interpreting the data as organic sulphur compounds may release sulphide under the test conditions.
<b>Principle of method.</b>	The sulphide ion reacts with para-aminodimethylaniline and ferric chloride to form methylene blue. Ammonium phosphate is added before color comparison to remove the color due to the presence of ferric ion.
<b>Time required for analysis.</b>	A single sample for sulphide analysis takes approximately 30 minutes. Approximately 50 samples can be analyzed daily.
<b>Range of application.</b>	This method is applicable to samples containing 0.02 to 2.0 mg/l of sulphide; higher concentrations are determined by sample dilution.
<b>Standard deviation.</b>	Not established.
<b>Accuracy.</b>	Not established. Sulphide standards are perishable and therefore no long-term quality control standards are used.
<b>Detection criteria.</b>	0.01 mg/l
<b>Interferences and shortcomings.</b>	High sulphide levels of several hundred milligrams per liter inhibit the reaction. Sulphite and thiosulphate interfere if their concentrations are greater than 10 mg/l. Nitrite interferes at 0.5 mg/l. Mercaptans, organic disulphides and hydrogen sulphide give a similar color with methylene blue.
<b>Minimum volume of sample.</b>	180 ml.
<b>Preservation and sample container.</b>	Samples are preserved by adding 2 ml of 2N zinc acetate solution per liter of sample followed by dropwise addition of 5% sodium carbonate solution until precipitation is complete.

**Safety  
considerations.**

Hydrogen sulphide is an extremely toxic gas. Low concentrations of the range that may be encountered in a laboratory environment may produce dizziness, headache, nausea and lassitude. Work should be carried out in a well ventilated fumehood.

## SULPHIDE

### Methylene Blue Spectrophotometric Method D

#### 1. Introduction

The colorimetric method is based on the reaction which takes place, under suitable conditions between p-aminodimethylaniline, ferric chloride and sulphide ion, resulting in the formation of methylene blue. Ammonium phosphate is added before color comparison to remove the color due to the presence of ferric ion.

#### 2. Interferences and Shortcomings

Sulphite and thiosulphate interfere if their concentrations are greater than 10 mg/l. Nitrite interferes at 0.5 mg/l. Hydrogen sulphide, mercaptans and organic disulphides give a similar color with methylene blue. Sulphide at very high levels (several hundred mg/l) inhibits the reaction.

#### 3. Apparatus

- 3.1. Folin digestion tubes, 50 ml.
- 3.2. UV-visible Spectrophotometer, Beckman Model 26 with sipper system and absorption cell inside unit.
- 3.3. Pipettes
- 3.4. Burette, 50 ml.
- 3.5. Erlenmeyer flasks, 250 ml.
- 3.6. Dropper bottles.
- 3.7. Volumetric flasks.

#### 4. Reagents

- 4.1. Zinc acetate dihydrate ( $(\text{CH}_3\text{COO})_2\text{Zn} \cdot 2\text{H}_2\text{O}$ ), crystals, reagent grade.
- 4.2. Sodium carbonate ( $\text{Na}_2\text{CO}_3$ ), anhydrous, powder, reagent grade.
- 4.3. p-Aminodimethylaniline oxalate, reagent grade.
- 4.4. Sulphuric acid ( $\text{H}_2\text{SO}_4$ ), concentrated, reagent grade.
- 4.5. Ferric chloride ( $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ ), hexahydrate, reagent grade.

- 4.6. di-Ammonium hydrogen orthophosphate ((NH<sub>4</sub>)<sub>2</sub>HPO<sub>4</sub>), powder, reagent grade.
- 4.7. Sodium sulphide nonahydrate (Na<sub>2</sub>S.9H<sub>2</sub>O), reagent grade.
- 4.8. Iodine, (I<sub>2</sub>), reagent grade.
- 4.9. Hydrochloric acid (HCl), concentrated, reagent grade.
- 4.10. Lead Perchlorate Solution (0.1M), prepared reagent bought from Orion.
- 4.11. **Zinc Acetate Solution (0.4N)**  
Dissolve 38.0 g zinc acetate dihydrate in distilled water and dilute to 1 liter.  
This solution is used for sample preservation.
- 4.12. **Sodium Carbonate Solution**  
Dissolve 5 g of sodium carbonate in distilled water and dilute to 100 ml.
- 4.13. **Amine-Sulphuric Acid Stock Reagent**  
Dissolve 26.6 g p-aminodimethylaniline oxalate in a cold mixture of 50 ml concentrated sulphuric acid and 20 ml distilled water. Cool and dilute to 100 ml with distilled water. Store in a dark bottle.
- 4.14. **Amine-Sulphuric Acid Reagent**  
Dilute 25 ml amine-sulphuric stock solution to 1 liter with 1:1 sulphuric acid.  
Store in a dark bottle.
- 4.15. **Ferric Chloride Solution**  
Dissolve 100 g ferric chloride in distilled water and dilute to 100 ml.
- 4.16. **di-Ammonium Hydrogen Orthophosphate Solution**  
Dissolve 50 g di-ammonium hydrogen orthophosphate in distilled water and dilute to 100 ml.
- 4.17. **Stock Sulphide Solution**  
In a volumetric flask dissolve 7.5 g of sodium sulphide nonahydrate, in distilled water and dilute to 1 liter.
- 4.18. **Standard Sulphide Solution**  
Dilute 20 ml of the stock solution to 1 liter with distilled water and standardize with lead perchlorate solution in a volumetric flask.  
**Standardization**  
Titrate an aliquot of sulphide stock solution to the inflection endpoint. An Orion meter with a specific ion electrode are used to monitor the titration.
- 4.19. **Working Sulphide Standards**  
Dilute 0, 0.5, 1.0, 2.0, 3.0, 4.0 and 5.0 ml sulphide standard solution to 50 ml in Folin digestion tubes.

## 5. Procedure

- 5.1. Pipette an aliquot of sample, blank and standards, into a Folin digestion tube and dilute to 50 ml.
- 5.2. Add 0.5 ml amine-sulphuric acid reagent and mix. Then add 0.1 ml ferric chloride solution and mix.
- 5.3. After 1 minute add 1.5 ml di-ammonium hydrogen orthophosphate solution and mix. Centrifuge to bring down any precipitate.
- 5.4. Read absorbances of the standards and sample against the reagent blank at 600 nm and plot a standard curve for  $\mu\text{g}$  sulphide against absorbance.

## 6. Calculation and Reporting

Determine hydrogen sulphide concentrations using the following equation:

$$\text{mg/l H}_2\text{S} = \frac{a}{b} \times 1.063$$

Where:

a =  $\mu\text{g}$  sulphide in sample as read from the calibration curve

b = volume of sample used in ml

Report values of 0 - 0.02 as less than 0.02 mg/l and report all other values to the nearest 0.02 mg/l.

## 7. Precision and Accuracy

Duplicates are not currently run. Quality Control solutions are not used, as hydrogen sulphide standards are unstable and therefore standards are not accurate.

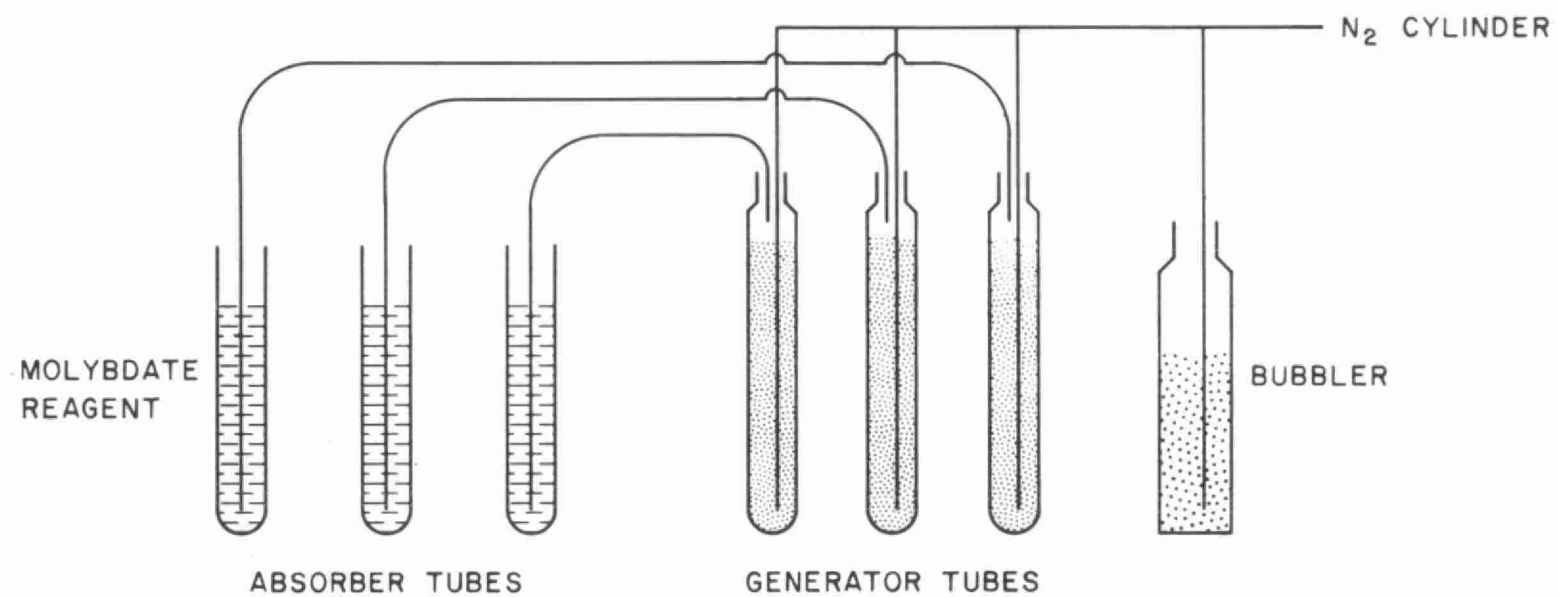


FIGURE 1 — GAS DISPLACEMENT-MOLYBDATE METHOD FOR SULPHIDE DETERMINATION



## THE DETERMINATION OF SUSPENDED AIR PARTICULATE

Suspended particulate matter in the atmosphere is present naturally and as a result of anthropogenic activity. Natural sources include wind blown dust, volcanic ash, pollens and smoke and fly ash from forest fires. Man's activities have greatly increased levels of suspended particulate, with the highest concentrations found in industrial areas, expressways, downtown areas and in the vicinity of solid waste incinerators. Residential and rural locations report relatively low levels of air particulate matter. Siliceous material generally comprises the largest component of total suspended particulate; however, the nature of the material depends primarily upon location and it is therefore difficult to generalize.

Suspended particulate in the atmosphere can cause a reduction in visibility, alterations in sky illumination due to changes in the light scattering pattern, a decrease in the amount of solar radiation reaching the earth's surface, damage to vegetation and property and physiological reactions including allergies and irritation to the respiratory tract. Health agencies have determined that particles of less than  $5\text{ }\mu\text{m}$  in diameter can enter the respiratory tract and cause health problems. The accepted standard for total suspended particulate matter in the atmosphere in Ontario is  $120\text{ }\mu\text{g}/\text{m}^3$  for 24 hours, with a geometric mean of  $60\text{ }\mu\text{g}/\text{m}^2$  for 1 year.

### Sample Handling and Preservation

Samples are collected on a tared glass fibre filter allowing the collection of particulate having an aerodynamic diameter of less than  $100\text{ }\mu\text{m}$ . Samples are collected by means of a high-flow rate blower drawing air in at a rate of 40 - 60 cfm. Exposed filters are carefully removed from the holder, folded across the middle of the 25.4 cm side, placed in suitably marked envelopes and mailed to the laboratory. The sample should be kept dry at all times.

### Selection of Method

The High Volume glass fibre filter method has been accepted by most North American agencies as the standard medium for the determination of total suspended particulates, although other filters such as Whatman No. 41 and Microsorban may be used for special analyses.

## SUSPENDED AIR PARTICULATE

### High Volume Gravimetric Method

#### SUMMARY

<b>Matrix.</b>	This method is used for suspended particulate determinations in the atmosphere.
<b>Substance determined.</b>	The total weight of suspended particulate matter.
<b>Interpretation of results.</b>	The total weight of suspended particulate matter is expressed as $\mu\text{g}/\text{m}^3$ . In order to calculate the change in the flow of air through the filter, initial and final flow rate values are required and it is presumed that for calculation purposes the decrease in flow rate becomes linear with time. This, however, is usually not the case (see Interferences and Shortcomings). Air flow controllers are now replacing flow meters.
<b>Principle of method.</b>	Air is drawn through a preweighed glass fibre filter by means of a high-flow rate blower at such a rate as to allow particulates having diameters of less than $100\ \mu\text{m}$ to reach the filter. The exposed aerodynamic filter is conditioned at 50% relative humidity and reweighed. The mass concentration of suspended particulate matter in ambient air is calculated from the filter weight difference and the volume of air sampled.
<b>Time required for analysis.</b>	An average of 56 filters can be prepared, conditioned and analyzed per day.
<b>Range of application.</b>	1 - 600 mg which is equivalent to $0.5\ \mu\text{g}/\text{m}^3$ - $300\ \mu\text{g}/\text{m}^3$ of air assuming $2000\ \text{m}^3$ of air passed through the filter during the exposure period. If more than 600 mg of particulate is present, the filter can be overloaded and excess dust is lost.
<b>Standard deviation.</b>	For an average mass concentration of $112\ \mu\text{g}/\text{m}^3$ of suspended particulate matter, the standard deviation is $10\ \mu\text{g}/\text{m}^3$ , at $39\ \mu\text{g}/\text{m}^3$ the standard deviation is $6\ \mu\text{g}/\text{m}^3$ based on reweighed filters.
<b>Accuracy.</b>	The overall accuracy of the method is completely dependent on obtaining true flow rates. Since estimates of the actual flow rate changes are not available, no accuracy statement can be provided.
<b>Detection criteria.</b>	The detection criteria of the balance is $\pm 2\ \text{mg}$ .

**Interferences and shortcomings.**

Air flow can be restricted as a result of oily smog or high humidity. The final calculation is based on an average flow and if the flow is restricted at the beginning of the sampling run, then the final loading will be lower than the actual reported value. Changing atmospheric conditions can result in an error as high as 30% in the true concentration. Compensating voltage regulators are now being installed to maintain a constant air flow.

Whenever the concentration of particulate is abnormally high, a loss may occur due to weak adhesion of the particulates to the filter.

Collected particulate matter may be hygroscopic. For this reason exposed filters are conditioned at constant humidity prior to final weighing.

**Minimum volume of sample.**

The standard glass fibre filter is 25.4 cm x 20.3 cm with an exposed area of 22.9 cm x 17.8 cm. Filters down to 47 mm diameter can be used for special determinations, using Low Volume or Dichotomous samplers.

**Preservation and sample container.**

Filters should be placed in an envelope and stored in a cool, dry area.

**Safety considerations.**

Normal laboratory safety procedures should be followed.

## SUSPENDED AIR PARTICULATE

### High Volume Method

#### 1. Introduction

The High Volume sampler is used primarily for the measurement of total suspended particulate matter, but further information concerning the collected particulate can be gained by analysis of the filter for such parameters as total metals, sulphates, nitrates, and gross  $\alpha$  and  $\beta$  radiation.

The High Volume sampler used (Figures 1, 2 & 3) by the Ontario Air Quality Network is used and recommended by the Environmental Protection Agency (8.2, 8.4). Air is drawn into a covered housing (Figure 2) by means of a high-flow rate blower at a rate of 19 - 29 l/sec. The housing allows only suspended particles having an aerodynamic diameter of less than 100  $\mu\text{m}$  to pass through to the filter. This technique measures the total weight of particles between 100  $\mu\text{m}$  and 0.3  $\mu\text{m}$ . The exposed filter is conditioned and reweighed and the difference in weights represents the air particulate matter trapped on the filter.

#### 2. Interferences and Shortcomings

Oily particulates such as photochemical smog and wood smoke cause a rapid drop in air flow at a non-uniform weight. Dense fog or high humidity causes the filter to become too wet resulting in a reduction in air flow through the filter. Glass fibre filters are comparatively insensitive to changes in relative humidity, whereas collected particulate may be hygroscopic (8.4). The latter is overcome by weighing the filter after conditioning in a constant humidity cabinet for a fixed period.

The final calculation assumes that the decrease in flow rate is linear with time. This, however, is not always the case since atmospheric conditions and the size of particulate impinging on the filter may restrict air flow. This problem may be overcome by compensating voltage regulators which produce a constant air flow.

#### 3. Apparatus

- 3.1. Humidity cabinet, maintained at 20 - 30°C and less than 50% relative humidity or a constant humidity room.
- 3.2. Balance, analytical, with sensitivity of 0.1 mg, equipped with weighing chamber designed to handle unfolded 25.4 cm x 20.3 cm filters.
- 3.3. Light source, light table similar to type used for viewing X-ray films.
- 3.4. Numbering device, for printing identification numbers on edges of filters.
- 3.5. Hi-Volume sampler, and shelter (Figures 1, 2 & 3).

- 3.6. Filter, glass fibre, having a collection efficiency of at least 99% for suspended particulates ranging in diameter from 100  $\mu\text{m}$  to 0.3  $\mu\text{m}$ . The Gelman A/E flash fired filter is one such filter. If a more detailed analysis of the particulate is required, a filter must be chosen which has an acceptably low control background of the pollutant being measured. In this case blank measurements are required to determine background levels of these pollutants.

#### 4. Reagents

- 4.1. Magnesium nitrate ( $\text{Mg}(\text{NO}_3)_2$ , reagent grade powder (for constant humidity cabinet).

4.2. Magnesium Nitrate Solution

Prepare a saturated magnesium nitrate solution using distilled water (for constant humidity cabinet).

**NOTE:** Reagents 4.1 and 4.2 are not required if a constant humidity room is available.

#### 5. Procedure

- 5.1. Inspect each filter visually. Place any suspect filter on light table and inspect for pinholes. Discard filters having visual imperfections. Stamp approved filters with lot, station and filter number.
- 5.2. Allow filter to equilibrate to balance room conditions for a minimum of 24 hours.
- 5.3. Weigh filters to nearest mg and record tare weight and filter identification number.
- 5.4. Without folding, place filter into its original box and deliver to field station. Field station operator will load Hi-Vol sampler, expose for 24 hours and return filter to the laboratory.
- 5.5. Condition the returned exposed filter for 16 hours or overnight in a constant humidity room or in a constant humidity cabinet which is maintained at approximately 50% humidity by means of a saturated magnesium nitrate solution.
- 5.6. Weigh conditioned filter and record loaded weight to nearest mg.

#### 6. Calculation and Reporting

Total suspended particulate reported as  $\mu\text{g}/\text{m}^3$  in air is calculated as follows:

$$\text{SP} = \frac{(w_2 - w_1) \times 10^6}{V}$$

Where:

SP = mass concentration of suspended particulates in  $\mu\text{g}/\text{m}^3$

$w_1$  = initial weight of filter in g

w<sub>2</sub> = final weight of filter in g  
v = air volume sampled in m<sup>3</sup>  
10<sup>6</sup> = conversion of g to µg

Results are expressed to the nearest µg/m<sup>3</sup> from 1 - 1000 µg/m<sup>3</sup>.

## 7. Precision and Accuracy

Based on collaborative testing, the relative standard deviation for single analyst variation is 3% (8.4). For an average mass concentration of 112 µg/m<sup>3</sup> suspended air particulates, the standard deviation is 10 µg/m<sup>3</sup>. At 39 µg/m<sup>3</sup> the standard deviation is 6 µg/m<sup>3</sup> based on reweighed filters.

The accuracy with which the sampler measures the true average concentration depends upon the constancy of the rate of airflow through the sampler. The airflow rate is affected by the concentration and the nature of the dust accumulating on the filter and by atmospheric conditions. As a result, the error in the measured average concentration may be in excess of 30% of the true concentration during the 24 hour sampling period.

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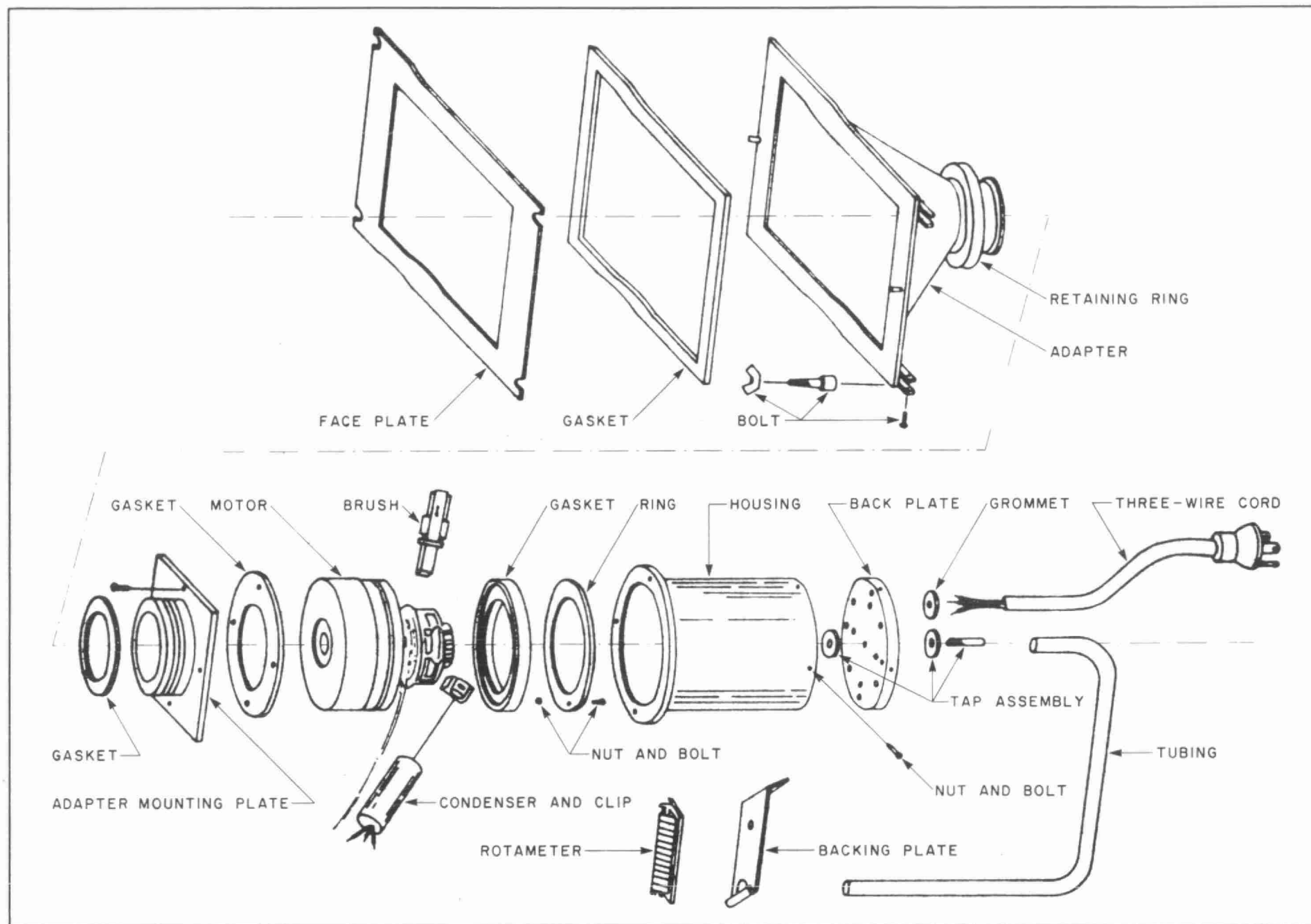


FIGURE 1 — EXPLODED VIEW OF TYPICAL HI-VOLUME SAMPLER PARTS (8.4)

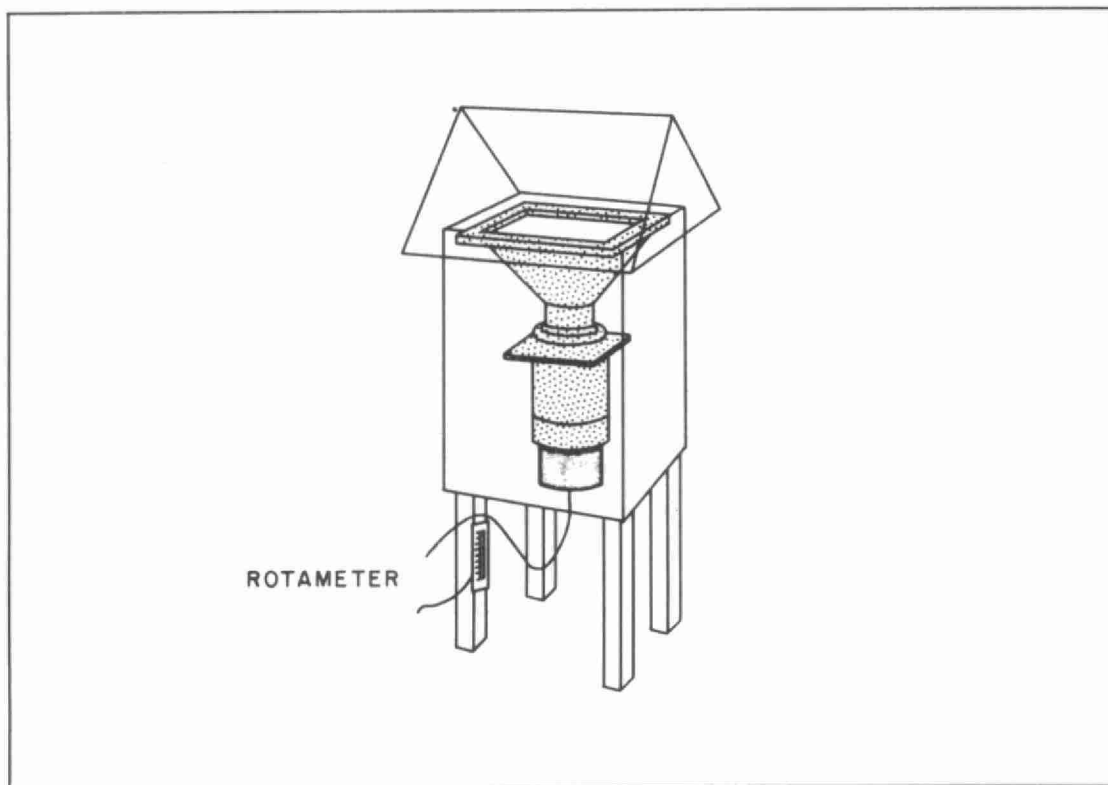


FIGURE 2 — ASSEMBLED SAMPLER AND SHELTER (8.4)

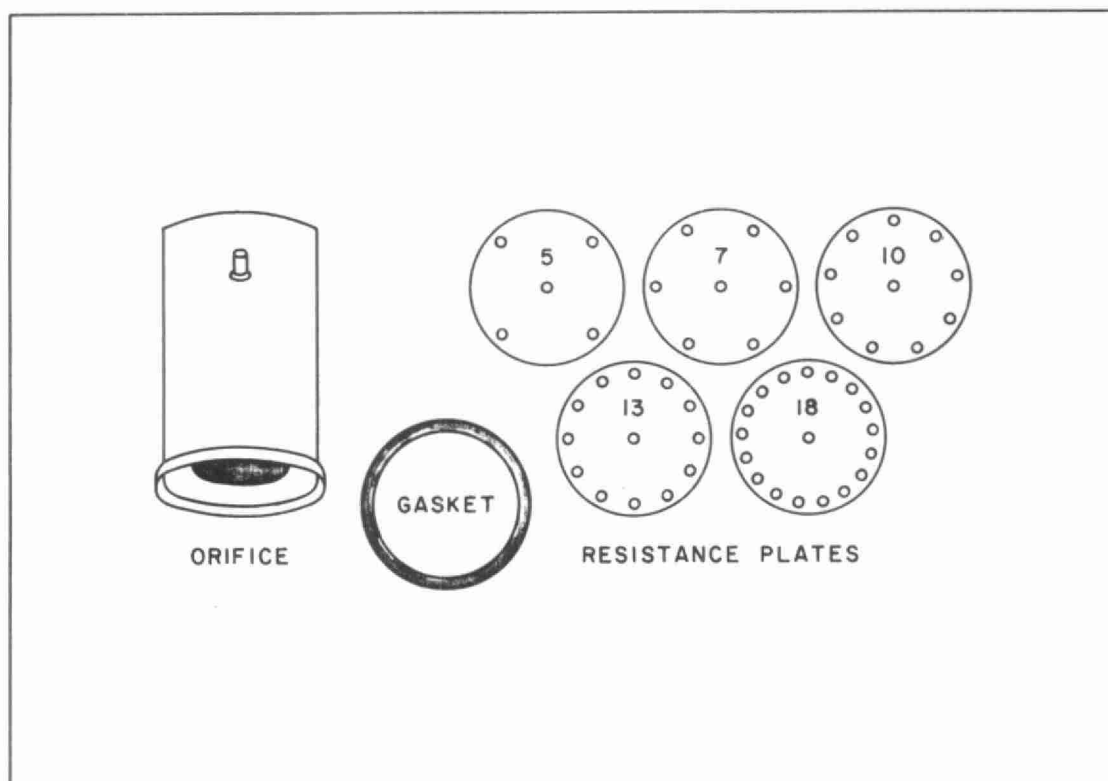


FIGURE 3 — ORIFICE CALIBRATION UNIT (8.4)



## THE DETERMINATION OF TOLUENE-2, 4-DIISOCYANATE

Toluene-2,4-diisocyanate (TDI), also referred to as meta-tolylene diisocyanate, is a clear, faintly yellow liquid with a sharp, pungent odour. Its molecular weight is 174.15; melting point is 19.5 to 21.5°C; boiling point is 251°C at 760 mm pressure. TDI reacts with water producing carbon dioxide and is soluble in ether, acetone, benzene, carbon tetrachloride and other solvents. Concentrated alkaline compounds, such as sodium hydroxide, may cause run-away polymerization.

TDI serves as the monomer in the manufacture of polyurethane foams and other elastomers which are widely used in commercial and industrial applications, such as insulating and packing materials. TDI is highly toxic by ingestion and inhalation; it is also irritating to eyes, skin and respiratory tract, causing allergic eczema and bronchial asthma.

The Ontario Occupational Health Guidelines allow maximum TDI concentrations (Threshold Limit Value) at 0.02 ppm (v/v), or 120  $\mu\text{g}/\text{m}^3$  air, for an 8 hour work day.

### Sample Handling and Preservation

#### Air

Air is passed at predetermined rates and volumes through Tenax sampling tubes where TDI is adsorbed on Tenax resin. The TDI is then desorbed from the resin at room temperature by pouring 10 ml n-hexane through the vertically positioned sample tubes. The resulting solutions of TDI are shipped to the laboratory in glass stoppered containers taking care to protect from light and heat. Analysis should be carried out within a 12-hour period after sample collection.

#### Solids

Aliquot samples, finely divided, are extracted with n-hexane. Only dry (non-aqueous) samples can be considered for analysis since water reacts readily with TDI.

### Selection of Method

A gas chromatographic method has been developed for separating TDI from other organic compounds in the sample. After desorption of TDI from the Tenax resin, the compound passed through the gas chromatographic column is burned in an air-hydrogen flame of the flame ionization detector and the TDI response is measured and recorded. This method is rapid, sensitive and allows the simultaneous measurement of a variety of compounds. A similar gas chromatographic method has been published, in which an electron capture detector is used. However, this detector was found to offer no significant advantage over the flame ionization detector.

Most other analytical methods involve colorimetric or spectrophotometric measurements of compounds produced from reactions of TDI with suitable reagents. These methods are invariably more complex and/or time-consuming than direct gas chromatography.

One colorimetric method for TDI is based upon hydrolysis to the corresponding diamine, diazotization, coupling to N-1-naphthylethylenediamine and color measurement at 550 nm. A high pressure liquid chromatographic method involves the conversion of TDI with a reagent containing N-4-nitrobenzyl-N-n-propylamine to form stable urea derivatives, which are chromatographed in a pellicular silica packed column with a hexane-ethanol solvent program and measured by a UV detector. Another analytical method for TDI employs p-dimethylaminobenzaldehyde (Ehrlich's Reagent) as a coupling agent to produce (in the presence of acetic acid) a bright yellow color which provides the basis for a colorimetric determination. Also, the reaction of isocyanates with p-aminoazobenzene resulting in a color change to orange-yellow has been used for the colorimetric determination of TDI.

## TOLUENE-2, 4-DIISOCYANATE

### Gas Chromatographic Method

#### SUMMARY

<b>Matrix.</b>	This method is used on air or (dry) solid samples.
<b>Substance determined.</b>	Toluene-2, 4-diisocyanate ( $\text{CH}_3\text{C}_6\text{H}_4(\text{NCO})_2$ ).
<b>Interpretation of results</b>	Results are reported in $\mu\text{g}/\text{m}^3$ or $\mu\text{g}/\text{kg}$ solids.
<b>Principle of method.</b>	Toluene-2, 4-diisocyanate (TDI) concentrations in air samples are determined by passing the samples through Tenax sampling tubes, extracting the adsorbed TDI from the Tenax resin with n-hexane and analyzing the extracts by gas chromatography. A gas chromatographic column is used to separate TDI from other organic compounds in the sample and the TDI is determined by a flame ionization detector. Solid samples are extracted with n-hexane and the extracts subjected to the same analytical procedure.
<b>Time required for analysis.</b>	Several samples may be tested in one day.
<b>Range of application.</b>	0.004 ppm (v/v) or $28.5 \mu\text{g}/\text{m}^3$ air is the lower detection limit.
<b>Standard deviation.</b>	Results of replicate analyses of TDI in solutions of n-hexane should not differ by more than 10% of the mean.
<b>Accuracy.</b>	Not yet determined.
<b>Detection criteria.</b>	0.004 ppm (v/v) or $28.5 \mu\text{g}/\text{m}^3$ air
<b>Interferences and shortcomings.</b>	Owing to its reactive isocyanate groups, TDI vapor in air possesses limited stability and at high humidity levels, deterioration occurs fairly rapidly. When adsorbed from the air in Tenax sampling tubes, TDI concentrations may decrease slightly within hours at room temperature.
<b>Minimum volume of sample.</b>	500 ml air or 50 g solids.

**Preservation and  
sample container.**

Samples are taken in Tenax sampling tubes. The TDI in air is adsorbed on the Tenax resin and then desorbed with n-hexane. The hexane extracts are shipped to the laboratory in glass stoppered containers and protected from heat and light. Analysis should be done within 12 hours.

**Safety  
considerations.**

Inhalation of TDI vapor and contact with eyes and skin should be avoided since TDI is a highly toxic chemical.

## **TOLUENE-2, 4-DIISOCYANATE**

### **Gas Chromatographic Method**

#### **1. Introduction**

Toluene-2, 4-diisocyanate (TDI) concentrations in samples are determined by passing air samples through Tenax sampling tubes, extracting the adsorbed TDI from the Tenax resin with n-hexane and analyzing the extracts with a gas chromatograph. A gas chromatographic column separates TDI from other organic compounds in the sample and the eluted TDI is burned in the air-hydrogen flame of a flame ionization detector. A gas chromatogram is produced on which peak heights (or peak areas) are proportional to the concentrations of organic compounds in the sample.

#### **2. Interferences and Shortcomings**

Owing to its reactive isocyanate groups, TDI vapor in air possesses limited stability and at high humidity levels, deterioration occurs fairly rapidly. When adsorbed from the air in Tenax sampling tubes, TDI concentrations can decrease slightly within hours at room temperature. Once dissolved in certain organic solvents, such as n-hexane, TDI is more stable. However, it is recommended that TDI solutions be stored for only short periods of time prior to analysis and that analyses be carried out preferably on the same day as sample collection.

#### **3. Apparatus**

- 3.1. Gas chromatograph equipped with flame ionization detector and an appropriate recorder.
- 3.2. Column, 2 m length and 2 mm inside diameter (ID) stainless steel, packed with 3% OV-17 on Chromosorb G, 80-120 mesh, conditioned for approximately 12 hours at 190°C with a nitrogen gas flow rate of 20 ml/min.
- 3.3. Sampling tubes, 15 cm by 1.3 cm, glass, packed with Tenax, 30-60 mesh, pre-purified and conditioned.
- 3.4. Air sampling pump with Teflon diaphragm, or equivalent, which does not adsorb or desorb organic vapors.
- 3.5. Flow meter, to measure sampling flow rate.
- 3.6. Glass containers with glass stoppers suitable for shipping TDI solutions.
- 3.7. Wrapping paper, non translucent.

#### 4. Reagents

- 4.1. Hydrogen ( $H_2$ ), ultra-high purity, 99.99%  $H_2$  minimum.
- 4.2. n-hexane ( $CH_3(CH_2)_4CH_3$ ), chromatographic purity.
- 4.3. Clean air, total hydrocarbon content of less than 0.1 ppm (v/v) as methane.
- 4.4. Nitrogen ( $N_2$ ), ultra-high purity, 99.99%  $N_2$  minimum.
- 4.5. Toluene-2, 4-diisocyanate ( $(CH_3C_6H_3(NCO)_2$ ), chromatographic purity.
- 4.6. Column packing material OV-17.
- 4.7. Column packing material Chromosorb G, 80-120 mesh.
- 4.8. Acetone, chromatographic purity.
- 4.9. Tenax, adsorbent, 30-60 mesh.
- 4.10. Anisaldehyde ( $C_8H_8O_2$ ), chromatographic purity.
- 4.11. **TDI Solution (for conditioning column).**

Dissolve 0.005 g TDI in n-hexane to a total volume of 10 ml.

#### 4.12. Calibration Standards

Prepare TDI solution in n-hexane at suitable concentration levels (eg. 2, 4, 8, 12 and 16 mg/l).

#### 4.13. Quality Control External Standard (20 mg/l)

Dilute 1  $\mu$ l anisaldehyde in 50 ml n-hexane. Other suitable concentrations of this standard may be prepared as required. This solution is used for recognizing changes in detector response (caused by the instability of TDI in the calibration standards) and sensitivity loss of the detector and for indicating the consistency of retention time.

#### 5. Procedure

##### 5.1. Sampling

- 5.1.1. To purify the Tenax used for TDI adsorption, thoroughly rinse with acetone, and then with n-hexane. Condition at  $140^{\circ}$ - $150^{\circ}$ C for 12-15 hours. Treat a sampling tube containing the purified Tenax with n-hexane at room temperature. Test the resulting extract by GC for the presence of interfering compounds.
- 5.1.2. Pass air to be sampled at a pre-determined rate and volume through Tenax sampling tubes.

- 5.1.3. Without delay, pour 10 ml n-hexane through the vertically positioned sampling tubes to desorb any adsorbed TDI from the resin. Ship these solutions to the laboratory in glass stoppered containers. Protect from heat and light. Analyze within 12 hours.

## 5.2. Gas Chromatograph Analysis

### 5.2.1. Operating Conditions

Column temperature: 120 °C (isothermal)  
Detector temperature: 250 °C  
Injector temperature: 175 °C  
Nitrogen carrier gas flow rate: 55 ml/min.  
Hydrogen carrier gas flow rate: 30 ml/min.  
Clean air flow rate: 300 ml/min.  
Sample size: 5 µl

- 5.2.2. Condition column with repeated injections (about 20 times) of 10 µl volumes of the TDI solution (reagent 4.11). The retention time under the above conditions is 10.8 minutes.

- 5.2.3. Calibration standards are injected into the GC and the peak heights recorded. Response factors are calculated for each concentration level according to 6.1.

- 5.2.4. Samples are analyzed as in 5.2.3 except that the sample is substituted for the standard.

## 6. Calculation and Reporting

- 6.1. Response factors for the calibration standards are calculated as follows:

$$RF = \frac{\text{Peak height (mm)}}{\text{TDI weight (ng)}}$$

- 6.2. The concentration of TDI in air is calculated from its gas chromatographic peak as follows:

$$c = \frac{p \times v_h}{RF \times v_i \times v_a}$$

Where:

c = concentration of TDI in air (ng/m<sup>3</sup>)  
p = peak height (mm)  
RF = response factor as calculated in 6.1  
v<sub>h</sub> = volume of n-hexane extract (µl)  
v<sub>i</sub> = volume of injected extract (µl)  
v<sub>a</sub> = volume of air (m<sup>3</sup>)

## 7. Precision and Accuracy

Results of replicate analyses of TDI solutions of n-hexane should not differ by more than 10% of the mean.

## 8. Bibliography

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- 8.5. Dunlap et al; Determination of Isocyanates in Working Atmospheres by High Speed Liquid Chromatography; Anal. Chem.; Vol. 48(3), p. 497; 1976.
- 8.6. Robinson; Atmospheric Determination of Tolyene Diisocyanates; Ind. Hyg. J., p. 228; 1962.
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## THE DETERMINATION OF TRIAZINE HERBICIDES

Triazine herbicides have been used as selective herbicides for corn, cotton, sorghum, sugar cane and several other crops. They are also used in the non-agricultural field for industrial weed and brush control. Triazines are highly selective due to their power of inhibiting photosynthesis in specific plants: it appears that tolerant plants are able to inactivate greater amounts of triazines than sensitive plants. Triazines are also highly persistent in soil and may interfere with normal crop rotation. Certain crops, such as tobacco and sugar beet, are highly susceptible to triazines.

The toxicity of triazines to wildlife, farm stock and humans appears to be low.

### Sample Handling and Preservation

#### Water Samples

Samples must be collected in 1 liter, amber, glass bottles with foil or Teflon-lined screw caps. These bottles are solvent rinsed prior to use and are labelled "For Pesticides and PCB Analysis Only". A minimum sample size of 500 ml is required and 800 ml is preferred.

#### Soil and Sediment Samples

Samples must be collected in solvent-rinsed, 16 oz, wide mouth, amber, glass jars with foil or Teflon-lined screw caps. Minimum sample size is 100 grams.

### Selection of Method

The routine analytical procedure for organochlorine pesticides was extended, with some modifications, to the analysis of herbicides. This method involves solvent extraction, column clean-up and gas chromatographic analysis with a Nitrogen-sensitive detector.

## TRIAZINE HERBICIDES

### Gas Chromatographic Method A - Water

#### SUMMARY

Matrix.	Surface water, domestic water, sewage, industrial waste and leachate samples.										
Substances determined.	The common triazine type herbicides: <table><tr><td>Prometone</td><td>Ametryne</td></tr><tr><td>Propazine</td><td>Sencor</td></tr><tr><td>Atrazine</td><td>Bladex</td></tr><tr><td>Prometryne</td><td></td></tr><tr><td>Simazine</td><td></td></tr></table>	Prometone	Ametryne	Propazine	Sencor	Atrazine	Bladex	Prometryne		Simazine	
Prometone	Ametryne										
Propazine	Sencor										
Atrazine	Bladex										
Prometryne											
Simazine											
Interpretation of results.	Results are reported in $\mu\text{g/l}$ or other units depending on concentrations.										
Principle of method.	A sample aliquot is solvent extracted, then dried, concentrated and cleaned up by alumina column chromatography to remove interferences. The cleaned up extract is examined by Gas Chromatography using a Nitrogen-specific detector and quantitation is by peak-height comparison.										
Time required for analysis.	Under optimum conditions, 8-10 samples may be analyzed for triazines in 2 days.										
Range of application.	From 0.05 $\mu\text{g/l}$ .										
Standard deviation.	Not available.										
Accuracy.	Not available.										
Detection criteria.	Detection criteria in $\mu\text{g/l}$ are as follows: Prometone: 0.05; Propazine: 0.05; Atrazine: 0.05; Prometryne: 0.05; Simazine: 0.05; Ametryne: 0.05; Sencor: 0.10; Bladex: 0.10.										
Interferences and shortcomings.	Other nitrogen-containing materials may interfere.										
Minimum volume of sample.	800 mls, obtained in specially prepared glass bottles.										

**Preservation and  
sample container.**

Only 1 liter, solvent rinsed, amber, glass bottles are acceptable. Caps should be foil or Teflon-lined to prevent contamination. No preservatives should be used. Samples should be refrigerated at 4°C to avoid bacterial degradation.

**Safety  
considerations.**

Solvents pose fire and exposure hazards; extreme care must be taken during their transport, storage and use.

## TRIAZINE HERBICIDES

### Gas Chromatographic Method A - Water

#### 1. Introduction

Triazine herbicides are determined by solvent extraction followed by column clean-up if necessary. The cleaned-up extract is analyzed by Gas Chromatography using a Nitrogen-sensitive detector.

#### 2. Interferences and Shortcomings

The use of a selective, Nitrogen-sensitive detector reduces the number of contaminants which may interfere with triazine determination.

#### 3. Apparatus

ALL GLASSWARE MUST BE RINSED THOROUGHLY WITH SOLVENT PRIOR TO USE.

##### 3.1. Extraction Procedure

- 3.1.1. Graduated cylinders, 100 ml, 1 liter.
- 3.1.2. Centrifuge tubes, 15 and 50 ml, Pyrex, graduated, with glass stoppers.
- 3.1.3. Pipette, Pyrex, 50 ml, graduated.
- 3.1.4. Rotary extractor.
- 3.1.5. Kontes blow-down apparatus.
- 3.1.6. Erlenmeyer flask, 500 ml.
- 3.1.7. Filter funnel, Pyrex.
- 3.1.8. Round-bottom flask, Pyrex, 300 ml, with 24/40 ground glass joint.
- 3.1.9. Rotary evaporator, Buchler.
- 3.1.10. Vacuum line.
- 3.1.11. Vortex evaporator, Buchler.

##### 3.2. Clean-up Procedure

- 3.2.1. Chromatographic column, Pyrex glass tubing, 28 cm x 6 mm I.D., with Teflon stopcock, 12/30 glass joint at top into which fits a 100 ml Pyrex reservoir.
- 3.2.2. Centrifuge tubes, Pyrex, 50 ml, graduated, with glass stopper.
- 3.2.3. Pasteur pipettes, glass, disposable.
- 3.2.4. Graduated cylinders, Pyrex, 25, 50 ml.
- 3.2.5. Beaker, Pyrex, 100 ml.

### 3.3. Gas Chromatographic Analysis

- 3.3.1. Gas chromatograph, Hewlett-Packard 5710A or equivalent equipped with Nitrogen-sensitive detector.
- 3.3.2. Column: Pyrex, 6 ft x 2 mm I.D.
- 3.3.3. Packing, 6% Carbowax on Chromosorb W.
- 3.3.4. Nitrogen-sensitive flame ionization detector.
- 3.3.5. Syringe, Hamilton, 10  $\mu$ l.
- 3.3.6. Volumetric flask, Pyrex, 100 ml, with glass stopper.

## 4. Reagents

### 4.1. Extraction Procedure

- 4.1.1. Hexane ( $\text{CH}_3(\text{CH}_2)_4\text{CH}_3$ ), residue-free, distilled in glass.
- 4.1.2. Dichloromethane ( $\text{CH}_2\text{Cl}_2$ ), residue-free, distilled in glass.
- 4.1.3. Sodium sulphate ( $\text{Na}_2\text{SO}_4$ ), anhydrous, extracted with dichloromethane, dried and stored at 130°C.
- 4.1.4. Isooctane,  $(\text{CH}_3)_3\text{CCH}_2\text{CH}(\text{CH}_3)_2$ .
- 4.1.5. Sodium hydroxide (NaOH), concentrated solution.
- 4.1.6. Glass fibre filter paper, solvent-extracted.

### 4.2. Clean-up Procedure

- 4.2.1. Alumina, basic, stored at 130°C.
- 4.2.2. Glass wool, solvent extracted.
- 4.2.3. Isooctane, distilled in glass, residue free.
- 4.2.4. As above in 4.1.1, 4.1.2 and 4.1.3.
- 4.2.5. Diethyl ether ( $\text{C}_2\text{H}_5\text{-O-C}_2\text{H}_5$ ).

### 4.3. Gas Chromatographic Analysis

- 4.3.1. Isooctane, distilled in glass, residue-free.
- 4.3.2. Hexane ( $\text{CH}_3(\text{CH}_2)_4\text{CH}_3$ ), residue-free, distilled in glass.
- 4.3.3. Stock Standard Solutions  
In volumetric flasks dissolve 0.01 g of each herbicide in acetone and dilute to 100 ml with acetone.
- 4.3.4. Working Standard Mixture (1  $\mu\text{g/ml}$ )  
In a volumetric flask dilute 1 ml stock standard solution to 100 ml with hexane.

## 5. Procedure

### 5.1. Extraction Procedure

- 5.1.1. Mark sample level on the outside of the bottle for determination of the water volume, which will be measured in a 1000 ml graduated cylinder, after the sample is extracted.
- 5.1.2. Add sodium hydroxide solution to make sample basic (pH 12).
- 5.1.3. Add 100 ml dichloromethane to the bottle and place on a rotary extractor.
- 5.1.4. Rotate for 15 minutes. Remove and allow sample to settle.
- 5.1.5. Using a 50 ml pipette attached to a vacuum line remove the lower solvent layer from the bottle.
- 5.1.6. Transfer solvent to Erlenmeyer flask.
- 5.1.7. Repeat twice more using 50 ml dichloromethane.
- 5.1.8. Dry combined extracts by passing through a filter funnel containing a glass fibre filter and 10 g sodium sulphate and collect in a 300 ml round bottom flask.
- 5.1.9. Rinse the flask twice with 15 ml aliquots of dichloromethane and pass through the sodium sulphate.
- 5.1.10. Rinse the filter cake with a further 10 ml aliquot of dichloromethane.
- 5.1.11. Place the round bottom flask on a rotary evaporator and evaporate to 5 ml under reduced pressure.
- 5.1.12. Transfer quantitatively to a 15 ml graduated centrifuge tube. Add 1.5 ml of isooctane and further reduce the volume to 0.5 ml using a Vortex evaporator. The sample is now ready for GC analysis.

### 5.2. Clean-up Procedure - If necessary

Alumina activity may vary, therefore each batch must be tested to determine the proper elution volumes.

- 5.2.1. Rinse column and reservoir with hexane.
- 5.2.2. Plug lower end with 0.5 cm glass wool.
- 5.2.3. Pour sufficient basic alumina into beaker and allow to cool.
- 5.2.4. Pack column with alumina to a height of 6 cm.
- 5.2.5. Transfer sample (5.1.12) to column using a Pasteur pipette.
- 5.2.6. Rinse sample container with a 1 ml aliquot of isooctane and transfer to column.
- 5.2.7. Elute with: a) 10 ml of 5% dichloromethane/hexane and b) 40 ml of 33% ether/hexane.
- 5.2.8. Fraction a) is discarded.
- 5.2.9. Collect fraction b) in a 50 ml centrifuge tube.
- 5.2.10. Add 1.5 ml isooctane to fraction b) to act as a keeper.
- 5.2.11. Using a Vortex evaporator, reduce the volume to 0.5 ml. Make up to the required volume with isooctane.

### 5.3. Gas Chromatographic Analysis

#### 5.3.1. Operating Conditions:

##### 5.3.1.1. Temperatures:

injector 250°C  
detector 300°C  
column 160 to 240°C at 4 degrees per minute

##### 5.3.1.2. Gas Flows:

Helium 10 ml/min.

##### 5.3.1.3. 6 ft x 2 mm column packed with Carbowax on Chromosorb W.

- 5.3.2. Allow gas chromatograph to equilibrate at required temperatures and gas flows. Inject 5 µl of standard mixture to obtain retention times.
- 5.3.3. Repeat 5.3.2. to check reproducibility.
- 5.3.4. Inject 5 µl aliquot of sample. Compare retention times of standard peaks to those of peaks in sample. If sample contains any corresponding peaks repeat the injection.
- 5.3.5. Detector response is linear allowing quantitation by peak height comparison.

The following herbicides are determined by this method: Prometone, propazine, atrazine, prometryne, simazine, ametryne, Sencor and Bladex.

### 6. Calculation and Reporting

Herbicide concentrations are determined by direct peak comparison. Results are reported in µg/l.

### 7. Precision and Accuracy

Not available.

### 8. Bibliography

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## TRIAZINE HERBICIDES

### Gas Chromatographic Method B - Soils and Sediments

#### SUMMARY

Matrix.	This method is used on soils and sediments.										
Substances determined.	The common triazine type herbicides: <table><tr><td>Prometone</td><td>Ametryne</td></tr><tr><td>Propazine</td><td>Sencor</td></tr><tr><td>Atrazine</td><td>Bladex</td></tr><tr><td>Prometryne</td><td></td></tr><tr><td>Simazine</td><td></td></tr></table>	Prometone	Ametryne	Propazine	Sencor	Atrazine	Bladex	Prometryne		Simazine	
Prometone	Ametryne										
Propazine	Sencor										
Atrazine	Bladex										
Prometryne											
Simazine											
Interpretation of results.	Results are reported in $\mu\text{g/g}$ or other units depending on concentrations.										
Principle of method.	A sample aliquot is solvent extracted, then dried, concentrated and cleaned up by alumina column chromatography to remove interferences. The cleaned up extract is examined by Gas Chromatography using a Nitrogen-specific detector and quantitation is by peak/height comparison.										
Time required for analysis.	Under optimum conditions, 8-10 samples may be analyzed for triazines in 2 days.										
Range of application.	From 0.05 $\mu\text{g/g}$ .										
Standard deviation.	Not available.										
Accuracy.	Not available.										
Detection criteria.	Detection criteria in $\mu\text{g/g}$ are as follows: Prometone: 0.05; Propazine: 0.05; Atrazine: 0.05; Prometryne: 0.05; Simazine: 0.05; Ametryne: 0.05; Sencor: 0.10; Bladex: 0.10.										
Interferences and shortcomings.	Other nitrogen-containing materials may interfere.										
Minimum volume of sample.	100 grams.										



**Preservation and  
sample container.**

Samples must be collected in 16 oz, wide mouth, amber glass jars with foil or Teflon-lined screw-caps. Samples should be refrigerated at 4°C.

**Safety  
Considerations.**

Solvents pose fire and exposure hazards; extreme care must be taken during their transport, storage and use.

## TRIAZINE HERBICIDES

### Gas Chromatographic Method B - Soils and Sediments

#### 1. Introduction

Triazine herbicides are determined by solvent extraction followed by column clean-up. The cleaned-up extract is analyzed by Gas Chromatography using a Nitrogen-sensitive detector.

#### 2. Interferences and Shortcomings

The use of a selective, Nitrogen-sensitive detector reduces the number of contaminants which may interfere with triazine determination. For soils and sediments, an alumina clean-up procedure removes many naturally-occurring interferences.

#### 3. Apparatus

ALL GLASSWARE MUST BE RINSED WITH SOLVENT PRIOR TO USE.

##### 3.1. Extraction Procedure

- 3.1.1. Top-loading balance, sensitive to 0.01 gram.
- 3.1.2. Beaker, Berzelius, 300 ml.
- 3.1.3. Sonifier Cell Disruptor.
- 3.1.4. Kontes vacuum filtration assembly including: Buchner funnel, 150 ml with sintered glass disc (porosity: coarse) and 24/40 inner joint and vacuum adapter.
- 3.1.5. Separatory funnel: 250 ml, cylindrical, with 24/40 outer joint at top, and Teflon stopcock at bottom.
- 3.1.6. Separatory funnel: 2 liter, with Teflon stopcock and Teflon stopper.
- 3.1.7. Boiling flask: 250 ml, round-bottom, 24/40 joint.
- 3.1.8. Buchler rotary evaporator.
- 3.1.9. Filter funnel, Pyrex.
- 3.1.10. Volumetric flask, Pyrex, 100 ml, with glass stopper.
- 3.1.11. Centrifuge tube, Pyrex, 15 ml, graduated, glass stoppered.
- 3.1.12. Vortex evaporator, Buchler.

##### 3.2. Clean-up Procedure

- 3.2.1. Chromatographic column: Pyrex glass tubing, 28 cm x 6 mm I.D., with Teflon stopcock; 12/30 glass joint at top into which fits a 100 ml reservoir.
- 3.2.2. Beaker: Pyrex, 100 ml, graduated.

- 3.2.3. Centrifuge tubes: Pyrex, 15 ml, graduated.
- 3.2.4. Pasteur pipettes: glass, disposable.
- 3.2.5. Graduated cylinders: Pyrex, 50 ml.
- 3.2.6. Vortex evaporator, Buchler.

### 3.3. Gas Chromatographic Analysis

- 3.3.1. Gas chromatograph, Hewlett-Packard 5800, with N/P detector or equivalent.
- 3.3.2. Column: Pyrex, 6 ft x 2 mm I.D.
- 3.3.3. Packing: 6% Carbowax on Chromosorb W.
- 3.3.4. Nitrogen-sensitive flame ionization detector.
- 3.3.5. Syringe, Hamilton, 10  $\mu$ l.

## 4. Reagents

### 4.1. Extraction Procedure

- 4.1.1. Acetone: residue free, distilled in glass.
- 4.1.2. Hexane: residue free, distilled in glass.
- 4.1.3. Distilled water: residue free.
- 4.1.4. Sodium sulphate: anhydrous, pre-extracted with dichloromethane, dried and stored at 130°C.
- 4.1.5. Celite 545: residue free.
- 4.1.6. Glass fibre filter paper: pre-extracted with dichloromethane.
- 4.1.7. Dichloromethane ( $\text{CH}_2\text{Cl}_2$ ), residue-free, distilled in glass.

### 4.2. Clean-up

- 4.2.1. Florisil: Floridin 60 - 100 PR activated at 130°C for at least one week before use and stored at 130°C.
- 4.2.2. As above in 4.1.2, 4.1.4 and 4.1.7.

#### 4.3. Gas Chromatographic Analysis

4.3.1. Isooctane (2,2,4-trimethylpentane), distilled in glass, residue free.

4.3.2. As above in 4.1.1 and 4.1.2.

##### 4.3.3. Stock Standard Solutions

In volumetric flasks dissolve 0.01 g of each herbicide in acetone and dilute to 100 ml with acetone.

##### 4.3.4. Working Standard Mixture (1 µg/ml)

In a volumetric flask dilute 1 ml of each stock standard solution to 100 ml with isooctane.

#### 5. Procedure

##### 5.1. Extraction

5.1.1. Determine water content (see: The Determination of Moisture Content).

5.1.2. Weigh the exact weight of sediment equivalent to 10.0 g dry-weight into a Berzelius beaker and add 50 ml acetone.

5.1.3. Extract by immersing Sonifier head in the sample suspension and vibrating at maximum speed for 3 minutes. The beaker should be cooled in a water bath to disperse the heat generated by the homogenizer. The increase and decrease in intensity should be gradual to prevent damage to the Sonifier. Rinse Sonifier head with 1 - 2 ml of acetone: let acetone drip into sample beaker.

5.1.4. Allow suspensions to settle before filtering.

5.1.5. Insert a glass fibre filter and a 2 cm layer of Celite 545 onto the sintered disc of the Buchner funnel and rinse the whole assembly with acetone.

5.1.6. Filter the supernatant through the Buchner filter into the cylindrical separatory funnel, with vacuum.

5.1.7. Add a further 50 ml of acetone to the remaining sediment and repeat extraction.

5.1.8. Filter the whole suspension through the Buchner funnel, rinsing all the sediment into the filter, using a minimum of acetone (15 ml at most) for rinsing.

5.1.9. Add the combined filtrates to a 2 liter separatory funnel containing 800 ml of tap water. The acetone to water ratio should be 1:8.

5.1.10. Extract three times with dichloromethane using one 100 ml portion and two 50 ml portions.

5.1.11. Dry dichloromethane the extracts through a filter funnel containing a glass fibre filter and sodium sulphate.

- 5.1.12. Collect the combined dry dichloromethane fractions in a round-bottom flask.
- 5.1.13. Evaporate to 70 - 80 ml on a rotary evaporator at 25°C. Adjust the vacuum rate of condensation to one drop per second.
- 5.1.14. Make up to 100 ml with dichloromethane in a volumetric flask.
- 5.1.15. Measure a 10 ml aliquot into a 15 ml graduated centrifuge tube.
- 5.1.16. Add 2 ml of isooctane and blowdown to 0.5 ml using a Vortex evaporator.
- 5.1.17. Make up to 1 ml with isooctane for clean-up.

## 5.2. Clean-up Procedure

Alumina activity may vary, therefore each batch must be tested to determine the proper elution volumes.

- 5.2.1. Rinse column and reservoir with hexane.
- 5.2.2. Plug lower end with 0.5 cm glass wool.
- 5.2.3. Pour sufficient basic alumina into beaker and allow to cool.
- 5.2.4. Pack column with alumina to a height of 6 cm.
- 5.2.5. Transfer sample to column using a Pasteur pipette.
- 5.2.6. Rinse sample container with a 1 ml aliquot of isooctane and transfer to column.
- 5.2.7. Elute with: a) 10 ml of 5% dichloromethane/hexane and b) 40 ml of 33% ether/hexane.
- 5.2.8. Discard fraction a).
- 5.2.9. Collect fraction b), containing the triazines, in a 50 ml centrifuge tube.
- 5.2.10. Add 2 ml isooctane to fraction b) to act as a keeper.
- 5.2.11. Using a Vortex evaporator, carefully evaporate to 0.5 ml. Make up to the required volume with isooctane for GC analysis.

## 5.3. Gas Chromatographic Analysis

### 5.3.1. Operating Conditions:

#### 5.3.1.1. Temperatures:

injector	250°C
detector	300°C
column	160 to 240°C at 4 degrees per minute

#### 5.3.1.2. Gas Flows:

Helium 10 ml/min.

#### 5.3.1.3. 6 ft x 2 mm I.D. column of 6% Carbowax on Chromosorb W.

- 5.3.2. Allow gas chromatograph to equilibrate at required temperatures and gas flows. Inject 5 µl of triazine standard mixture to obtain retention times.

- 5.3.3. Repeat 5.3.2. to check reproducibility.
- 5.3.4. Inject 5  $\mu$ l aliquots of sample. Compare retention times of standard peaks to those of peaks in sample. If sample contains any corresponding peaks repeat the injection.
- 5.3.5. Quantitate by comparing relative peak heights of sample and standards.

The following herbicides are determined by this method: Prometone, propazine, atrazine, prometryne, simazine, ametryne, Sencor and Bladex.

## 6. Calculation and Reporting

Herbicide concentrations are determined by direct peak height comparison. Results are reported in  $\mu$ g/g.

## 7. Precision and Accuracy

Not available.

## 8. Bibliography

- 8.1. U.S. Environmental Protection Agency. 1976. Analysis of Pesticide Residues in Human and Environmental samples. Environmental Toxicology Division, Health Effects Laboratory, Research Triangle Park, N.C.
- 8.2. U.S. Department of Health, Education and Welfare. 1965. Guide to the Analysis of Pesticide Residues - Volumes 1 and 2. Public Health Service, Bureau of State Services (Environmental Health), Office of Pesticides, Washington, D.C.

## THE DETERMINATION OF TURBIDITY

The demand for clear water by food processing manufacturing industries, as well as the general public, has created the need for a precise and sensitive technique for measuring low concentrations of fine suspended material in the water. The light scattering effect of such suspended materials is called turbidity. A turbidity measurement is an evaluation of the optical property of a sample which causes light to be scattered and absorbed rather than transmitted in straight lines through the sample.

In water samples, turbidity is a result of suspended clay, silt, finely divided organic and inorganic matter, plankton and other microscopic organisms. Turbidity cannot be successfully correlated with the weight concentration of suspended matter as the size, shape, refractive index and consequently the light scattering effect of suspended particles bear little relationship to their concentration and specific gravity.

### Sample Handling and Preservation

Glass, polyethylene or styrene containers are acceptable. Since the effects of preservation on suspended material are unpredictable and uncertain, preservatives are not recommended. Freezing should be avoided. Samples should be stored in darkness and examination within 24 hours of sampling is recommended.

### Selection of Method

In the past, the measurement of turbidity was performed using the Jackson candle turbidimeter, but the lower limit of 25 turbidity units imposed by this instrument requires that a secondary instrument be used to measure the much lower turbidities commonly encountered in modern water treatment processes. To accommodate low turbidity levels, the light scattering of the sample is measured at  $90^{\circ} \pm 30^{\circ}$  using a nephelometer (Method A) with Formazin turbidity standards. No instrument has been devised which duplicates the results obtained on the Jackson candle turbidimeter for all samples, since there is no direct relationship between Jackson candle turbidity and the light intensity scattered at  $90^{\circ}$ .

The turbidity test is therefore defined by the instrument and standards used as well as by the optical phenomenon being measured. Results are reported as FTU (Formazin turbidity units) rather than JTU (Jackson turbidity units).

**TURBIDITY**  
**Nephelometric Method**

**SUMMARY**

<b>Matrix.</b>	This method is used routinely for turbidity measurements on water samples.
<b>Substance determined.</b>	The optical property of a sample which causes light to be scattered and absorbed rather than transmitted in straight lines.
<b>Interpretation of results.</b>	Results are reported in FTU (Formazin turbidity units). Turbidity may not be correlated with suspended solids or compared with turbidity values of the same sample obtained using an instrument operating on different principles from the instrument used.
<b>Principle of method.</b>	Using a nephelometer calibrated to read in turbidity units, the light scattered at $90^{\circ} \pm 30^{\circ}$ by a sample is compared with the light scattered by a series of sealed standards which are calibrated against a primary Formazin standard.
<b>Time required for analysis.</b>	Each measurement requires approximately 3 minutes.
<b>Range of application.</b>	The range covered is from 0.2 to 1000 FTU. Dilution or concentration is not practiced.
<b>Standard deviation.</b>	For the 0.2 - 10.0 FTU range, standard deviations are 0.127 for 0 - 20% of the range; 0.247 for 20 - 50% of the range and 0.278 for 50 - 100% of the range. For the 10 - 100 FTU range, standard deviations are 1.12 for 0 - 20% of the range; 1.69 for 20 - 50% of the range and 1.54 for 50 - 100% of the range.
<b>Accuracy.</b>	Not available.
<b>Detection criteria.</b>	0.209 FTU.
<b>Interferences and shortcomings.</b>	Color due to dissolved substances which absorb light cause low results. Rapidly settling particles or gross debris may cause erroneous results.
<b>Minimum volume of sample.</b>	50 ml.



**Preservation and  
sample container.**

Preservatives should not be added. Samples should be kept in darkness and analysis within 24 hours is preferred. Glass, polyethylene or styrene bottles are acceptable.

**Safety  
considerations.**

If Formazin stock standard is prepared from methenamine and hydrazine sulphate, caution should be used since these reagents will cause severe burns.

## TURBIDITY

### Nephelometric Method A

#### 1. Introduction

The turbidimeter used is a nephelometer standardized against turbidity standards, and operating on the principle that light passing through a substance is scattered by any particulate matter present. The degree of light scattering is directly proportional to the degree of turbidity.

A focused light beam is directed upward through a cell containing the sample. A fraction of the light scattered at  $90^\circ \pm 30^\circ$  to the incident beam by the particulate matter in the sample is detected by a photomultiplier tube. The electrical signal produced is displayed on the instrument's meter which is calibrated to read directly in Formazin turbidity units (FTU). Calibration is accomplished with a standard suspension of Formazin polymer.

#### 2. Interferences and Shortcomings

Color resulting from dissolved substances which absorb light causes measured turbidities to be low.

Rapidly settling sediments or debris may cause erroneous results.

#### 3. Apparatus

3.1. Turbidimeter, Hach, model 2100A, (modified to provide sample stirring and capacitive damping during measurement) and assorted accessories.

3.2. Rack, suitable for holding sample cells upright and convenient to handle.

**NOTE:** Sample cells are initially cleaned with Decon-75 detergent solution, and are stored submerged in mild soap solution. Cells are rinsed thoroughly and inspected for freedom from particles before use. Detergent cleaning is repeated as required.

#### 4. Reagents

##### 4.1. Turbidity Free Water:

Pass distilled water through a membrane filter having a pore size of  $1.2 \mu$ . Discard the first 200 ml collected. If the filtrate shows a lower turbidity than the distilled water, use filtered water.

##### 4.2. Calibration Standards

The instrument is calibrated using the sealed standards provided in the Hach Turbidity Standards kit for Hach Turbidimeter model 2100A. The standards

are prepared from latex polymers of known size or from chlorobenzene solution and have turbidities of 0.61 FTU, 10 FTU, 100 FTU and 1000 FTU. These standards are calibrated against Formazin which is the primary standard turbidity material used by Hach.

**NOTE:** Formazin standards can be prepared from hydrazine sulphate and methenamine; however the solution is unstable after 1 day.

## 5. Procedure

REFER TO MANUFACTURER'S INSTRUCTION MANUAL FOR CLEANING AND OPERATING INSTRUCTIONS.

- 5.1. Turn on turbidimeter at least 2 hours prior to use. Set instrument on 0 - 1.0 FTU range.
- 5.2. Examine light apertures and top surface of lens system for accumulated dust. If necessary clean according to instruction manual.
- 5.3. If possible, arrange samples in order of increasing turbidity.
- 5.4. Remove sample cells from soaking solution and rinse thoroughly with tap water, followed by distilled water. Inspect cells for any remaining particles and remove such particles by gentle wiping with a Kim-Wipe. Carefully dry the exterior of the cell.

### 5.5. Calibration

- 5.5.1. Set meter to 10 scale, place 10 FTU standard in slot and cover. Adjust STANDARDIZE knob until it reads 10.
- 5.5.2. Set meter to 1 scale, place 0.61 FTU standard in slot and cover. (When using 0.61 standard ensure that tube is wiped clean with lens tissue.) This standard should read 0.59 - 0.63 FTU.
- 5.5.3. Set meter to appropriate scale and measure 100 FTU and 1000 FTU standards. Use cell riser. The 100 FTU standard should read 98 - 100 FTU and the 1000 FTU standard should read 980 - 1000 FTU.
- 5.5.4. If 0.61 FTU, 100 FTU and/or 1000 FTU standards do not read within the accepted range consult manufacturer's manual for adjustment procedure. Adjustments should not be required on a routine basis.

### 5.6. Measurement of Turbidity

- 5.6.1. Mix sample sufficiently to evenly disperse particulate matter throughout the sample.
- 5.6.2. Handling by the top only, rinse a sample cell twice with 10 ml portions of sample, and fill the cell to within 1.25 cm from the top. Carefully wipe the exterior of the cell to remove any liquid, dirt, or fingermarks.

- 5.6.3. Insert cell into the instrument and position the light shield-stirrer. Be sure that the light shield-stirrer is properly seated. Set STIRRER switch to the DISCHARGE position.
- 5.6.4. Using RANGE SELECTOR, select the measurement range which yields the maximum on-scale pointer deflection. Always use the cell riser on the 0 - 100 and 0 - 1000 FTU ranges.
- 5.6.5. Set STIRRER to READ position and allow the meter to come to a steady reading (10 - 15 seconds).
- 5.6.6. Read turbidity in FTU to the nearest 1% of full scale from the appropriate meter scale. Check the range setting before and after reading the meter to ensure that the reading is taken from the proper scale. Record range and reading.
- 5.6.7. Turn STIRRER to OFF, and wipe gently with a Kim-Wipe, to minimize cross contamination of samples. Put light shield-stirrer in its bracket, remove, empty, and rinse sample cell.
- 5.6.8. Repeat procedure for remaining samples.

## 6. Calculation and Reporting

Report turbidity results according to the following schedule.

Range (FTU)	Report to the nearest
0 - 1	0.01
1 - 10	0.1
10 - 100	1
100 - 1000	10

minimum reported turbidity; <0.2  
maximum reported turbidity; >1000

## 7. Precision and Accuracy

Since concentration range varies with sample, standard deviations are given in the following table:

Sample	Conc. Range	Standard Deviation		
		0-20%	20-50%	50-100%
River and lakes	0.2 - 10.0 mg/l	0.127	0.247	0.278
	10.0 - 100 mg/l	1.12	1.69	1.54
Drinking water	-	-	-	-

## 8. Bibliography

- 8.1. American Public Health Association, American Water Works Association and Water Pollution Control Federation (1971). Standard Methods for the Examination of Water and Wastewater. 13th edition, APHA, Washington, D.C. Section 163.
- 8.2. Duchrow, R.M. and Everhart, W.H. (1971). Turbidity measurement. Transactions of the American Fisheries Society, 100 (4): 682-690.
- 8.3. Hach, C. (no date). Introduction to Turbidity Measurement. Hach Chemical Co., Ames, Iowa.
- 8.4. Yoe, J.H. (1929). Photometric Chemical Analysis. Volume II, Nephelometry, John Wiley and Sons, London.

## THE DETERMINATION OF VINYL CHLORIDE

Vinyl chloride (chloroethene, chloroethylene, vinyl chloride monomer,  $\text{CH}_2=\text{CHCl}$ , VCM) is a colorless gas with a faintly sweet odor. Its molecular weight is 62.50; melting point is  $-160^\circ\text{C}$ ; boiling point is  $-14^\circ\text{C}$  at 760 mm pressure. It is slightly soluble in water and soluble in alcohol. VCM polymerizes in light or in the presence of catalysts. Major reaction products of VCM atmospheric degradation are formic acid, hydrogen chloride, carbon monoxide, formaldehyde, and ethylene, all of which are undesirable pollutants in the troposphere since they are irritant or corrosive substances. VCM is a suspected carcinogen and causes angiosarcomas in the liver of test animals when inhaled at concentrations greater than 50 ppm in air.

Vinyl chloride is widely used in industrial and manufacturing processes and consequently enter the surrounding environment. VCM serves as the basis for the production of polyvinyl chloride (PVC) thermoplastic resin which is used in electrical insulation, plumbing pipes, and wrapping materials. The vinyl chloride monomer (VCM) has been used in aerosol propellants for hair sprays, spray paints and pesticides. In Canada, aerosol products containing VCM have been banned.

VCM is commercially manufactured from naphtha, ethylene or acetylene, and involves oxychlorination, hydrochlorination, or dehydrochlorination and various combinations of these processes. Dichloroethane is usually first produced and then dehydrochlorinated to VCM either by thermal cracking or by the use of strong caustic soda.

VCM emissions to the atmosphere occur from monomer and polymer production processes, secondary fabrication processes of polyvinyl chloride resin, the production and release of VCM spray propellants, and the incineration of organic chlorinated waste materials.

The current Ontario 24-hour average ambient air quality guideline for VCM is 0.1 ppm v/v, or  $280 \mu\text{g}/\text{m}^3$  air. The Ontario Occupational Health Standard for VCM for a time weighted average (TWA) 8-hour exposure is 10 ppm v/v, or  $28 \text{ mg}/\text{m}^3$  air.

### Sample Handling and Preservation

#### Ambient Air

Two sampling procedures are used. Instantaneous grab samples taken over a short period of time are collected in polyester protected aluminized sample bags with a 1 to 40 liter capacity and equipped with snout flap and septum. Continuous samples taken over a longer period of time are obtained by adsorption of the contaminant to be measured on charcoal, activated at  $200^\circ\text{C}$  for 48 hours.

Analysis should be carried out as soon as possible after sample collection and exposure of sampling bags to heat and direct sunlight should be avoided. Sample bags may be reused after repeated evacuation and refilling with purified nitrogen or air. Adsorption tubes should be stored in the dark under refrigeration. Wrap a label around each tube and note direction of air flow through the tube, date, location, time on, time off, flow rate on and off, wind speed and direction and air temperature.

### Water

Aqueous samples should be collected in 1 liter glass bottles, tightly sealed with foil lined caps. No preservative is required but samples should be refrigerated while awaiting analysis.

### Soil

Soil and sediment samples of 20 to 200 g should be collected in pomade jars tightly sealed with foil lined caps, and then refrigerated until analysis.

### Vegetation

Vegetation samples of 20 to 200 g should be collected in polyethylene bags and then kept under refrigeration until analysis.

### **Selection of Method**

Bag samples are analyzed by gas chromatography after direct injection. Adsorption tube samples are subjected to desorption with carbon disulphide prior to injection into the gas chromatograph. Solid and vegetation samples are analyzed by gas chromatography by using headspace technique. Aqueous samples are analyzed by direct injection if feasible or by headspace analysis of the vapours in sealed bottles fitted with Teflon septa. Solid and vegetation samples are also analyzed by headspace technique.

VINYL CHLORIDE  
Gas Chromatographic Method

SUMMARY

Matrix.	This method is used for vinyl chloride determinations on air, vegetation, soil, surface water, drinking water, sewage, industrial waste and leachate samples.
Substance determined.	Vinyl chloride $\text{CH}_2 = \text{CHCl}$ .
Interpretation of results.	Results are reported as ppm (v/v) or $\mu\text{g}/\text{m}^3$ in air, or $\mu\text{g}/\text{l}$ in water, or $\mu\text{g}/\text{kg}$ in soil and vegetation.
Principle of method.	A gas chromatographic column is used to separate vinyl chloride from other organic compounds in the sample. Each compound is identified by its retention time (time required to pass through the column). Once eluted, the compound is burned in an air-hydrogen flame of a flame ionization detector (FID) and a gas chromatogram is produced in which the peak heights are proportional to the concentrations of the organic compounds in the sample.
Time required for analysis.	<p>Up to 18 air samples can be analyzed per day if gas sampling bags are used for sample collection (instantaneous method).</p> <p>Up to 7 samples can be analyzed per day if gas adsorption tubes are used for sample collection (continuous method).</p> <p>Soil, vegetation or water analysis requires approximately one half hour per sample.</p>
Range of application.	<p>Gas sampling bags: 0.03 ppm v/v or <math>80 \mu\text{g}/\text{m}^3</math> air is the lower detection limit.</p> <p>Gas adsorption tubes: 0.002 ppm v/v or <math>6 \mu\text{g}/\text{m}^3</math> air is the lower detection limit.</p> <p>Water: 100 <math>\mu\text{g}/\text{l}</math> is the lower detection limit for the direct injection technique; 0.2 <math>\mu\text{g}/\text{l}</math> is the lower detection limit for the headspace technique.</p> <p>Soil and vegetation: 2 <math>\mu\text{g}/\text{kg}</math> is the lower detection limit for the headspace technique.</p> <p>No upper limit problems for measuring concentrations of VCM have been experienced, although appropriate sample dilution prior to analysis may be required.</p>



<b>Standard deviation.</b>	Results of replicate analyses should not differ by more than 10% of the mean.
<b>Accuracy.</b>	Not yet determined. The major concern lies in the presence or absence of the compounds, not in their absolute amounts.
<b>Detection criteria.</b>	Gas sampling bags: 0.03 ppm v/v or 80 $\mu\text{g}/\text{m}^3$ air. Gas adsorption tubes: 0.002 ppm v/v or 6 $\mu\text{g}/\text{m}^3$ air. Water: 100 $\mu\text{g}/\text{l}$ for the direct injection technique; 0.2 $\mu\text{g}/\text{l}$ for the headspace technique. Soil and vegetation: 2 $\mu\text{g}/\text{kg}$ for the headspace technique.
<b>Interferences and shortcomings.</b>	Samples in bags have occasionally been found to contain traces of an unidentified compound with a retention time 94% of VCM. To avoid interference by this compound, a VCM retention time of greater than 3 minutes should be used.
<b>Minimum volume of sample.</b>	The gas sample bags should be at least half full to allow syringe samples to be taken. For gas adsorption tubes, 10 liters of air should pass through tubes although less may be sufficient for air samples taken in close proximity to emission sources.  For water samples, a minimum of 150 ml is needed (more for samples containing lower amounts of vinyl chloride).
<b>Preservation and sample container.</b>	Gas sample bags should be made of polyester protected aluminized barrier material. Gas adsorption tubes should be made of glass and contain activated charcoal. Sample bags may be stored at room temperature for 6 days without significant loss of sample. Adsorption tubes should be stored in the dark under refrigeration (maximum of 2 weeks). Samples should be analyzed as soon as possible.  Water samples are taken in glass bottles and are not preserved. Soil samples are collected in pomade jars. Vegetation samples are collected in plastic bags. Soil and vegetation samples are analyzed as collected in the field, therefore drying and grinding are not required. If not analyzed within a few hours, water, soil, and vegetation samples should be refrigerated after collection.
<b>Safety considerations.</b>	Carbon disulphide, used in desorption is very flammable and toxic and should therefore be carefully handled in either a fume hood or a well ventilated area. For vinyl chloride monomer the same precautions are required.

## VINYL CHLORIDE

### Gas Chromatographic Method

#### 1. Introduction

Vinyl chloride in air is sampled either with an air sample bag (instantaneous sampling) or with gas adsorption tubes (continuous sampling). Aliquots of sample taken from the air sample bags are injected directly into the gas chromatograph. Air samples collected in gas adsorption tubes involve a preliminary desorption of the VCM using carbon disulphide. Water, soil and vegetation samples are heated in sealed vessels and head space samples are taken and injected into the gas chromatograph. The separation is achieved by appropriate column conditions and detection/quantification is performed by a flame ionization detector. A recorder plots signal intensity against time and the peak height (or area) is proportional to the VCM concentration in the sample.

#### 2. Interferences and Shortcomings

Air sample bags are bulky and inconvenient for storage and shipment. However, bag samples permit direct analysis by gas chromatography although these samples may contain VCM at levels too low for direct measurement. Samples should be analyzed promptly as VCM may undergo condensation and reactions at the wall surfaces.

Samples have occasionally been found to contain traces of an unknown compound having a retention time of 94% that of VCM. By the EPA developed method, which has a VCM retention time of less than 1 minute, the peaks of both compounds remain unseparated resulting in high VCM values. To avoid this interference, a retention time greater than 3 minutes should be used.

Gas adsorption tubes are more convenient to transport and permit sample collection over a longer period of time. The concentration of VCM in the sampling tube allows the measurement of much lower VCM levels. The gas adsorption tubes, however, require an additional adsorption-desorption procedure.

Certain hydrocarbons (neopentane, butadiene) and Freons (dichlorodifluoromethane) have been reported to have elution characteristics similar to VCM and, therefore, should be considered at locations where these materials might be present.

#### 3. Apparatus

- 3.1. Gas chromatograph (GC) equipped with a flame ionization detector and an appropriate recorder.
- 3.2. Column, 2m long, 2mm inside diameter (ID), stainless steel, packed with "Porapak N" (80-100 mesh) conditioned for approximately 12 hours at 190°C with a nitrogen gas flow rate of 20 ml/min.

### 3.3. Optional gas chromatographic equipment:

- 3.3.1. Temperature programmer
  - 3.3.2. Dual columns with dual flame ionization detectors
  - 3.3.3. Backflush valve
  - 3.3.4. Integrator
- 3.4. Syringes, gas-tight with stainless steel hypodermic needles (18-22 guage)
  - 3.5. Syringes, microliter
  - 3.6. Pipette, 20 ml
  - 3.7. Test tubes, 25 ml, glass stoppered
  - 3.8. Ice bath
  - 3.9. Tube caps, Teflon

### Air Sampling

- 3.10. Gas sampling bags, aluminum layered polyester plastic, 1-40 liter capacity, equipped with snout, silicone rubber septum and gas valve. (Scotchpak bags have been found suitable and are available from J. W. Ellis Industries, Scarborough, Ontario or from Calibrated Instruments Inc., Ardsley, N. Y., U.S.A.) Bags may also be used for instrument calibration.
- 3.11. Tygon bubble tubing with Teflon tube insert (Figure 1).
- 3.12. Unidirectional valve, in sample line to bag (used to prevent sample loss when pump is switched off).
- 3.13. Air sampling pump, Teflon diaphragm or stainless steel bellow pump or equivalent. Pump must not adsorb or desorb organic vapors.
- 3.14. Air metering device, hypodermic needle or similar orifice. Orifice size depends on sampling period.
- 3.15. Bypass valve for sample flow rate adjustment.
- 3.16. Electric timer to shut off pump at end of sampling period.
- 3.17. Flowmeter, for sample flow rate measurement. (For a 22 liter bag and a 24 hour sampling period, use a flow rate of 15 ml/min; for a 30 min sampling period, a flow rate of 700 ml/min is required).
- 3.18. Gas sampling tubes, glass, nominal volume 500 ml, with Teflon core stopcocks, with provision for rubber septum attachment. Before use in calibration, determine volume of each tube by filling tube with distilled water and weighing. Gas sample tubes with glass core stoppers are not suitable since the grease used for lubrication may dissolve or desorb significant amounts of organic vapors.
- 3.19. Glass beads, 2 mm diameter.

- 3.20. Stainless steel tubing, 2 mm ID, thoroughly cleaned before use.
- 3.21. Pressure regulators for compressed air, hydrogen and nitrogen cylinders.
- 3.22. Lecture bottle septum assembly.
- 3.23. Air filter, Teflon, 5  $\mu$ m pore size, and Teflon or stainless steel filter holder.
- 3.24. Fold-back clips, 50 mm, for clamping sample bags.
- 3.25. Wet-test gas meter.
- 3.26. Cartridge containing Drierite or equivalent desiccant and 5A Molecular Sieve adsorbant for the removal of traces of moisture and hydrocarbons from carrier gas.
- 3.27. Sample inlet tubing, Teflon.
- 3.28. Gas adsorption sampling tubes, 250 mm long, 10 mm ID.

#### **Water, Soil and Vegetation Sampling**

- 3.29. Hypovials and caps.

#### **4. Reagents**

- 4.1. Hydrogen ( $H_2$ ), ultra high purity, 99.99%  $H_2$  minimum.
- 4.2. Methylene chloride ( $CH_2Cl_2$ ), for gas chromatography.
- 4.3. Clean air, total hydrocarbon content less than 0.1 ppm (as methane) as tested by gas chromatography.
- 4.4. Nitrogen ( $N_2$ ), ultra high purity, 99.99%  $N_2$  minimum.
- 4.5. Vinyl chloride ( $CH_2=CHCl$ ).
- 4.6. Methyl chloride ( $CH_3Cl$ ) and hydrocarbons to serve as gas chromatographic reference compounds.
- 4.7. Detergent, Alconox or equivalent.
- 4.8. Column packing material, Porapak N, 80-100 mesh.
- 4.9. Coconut charcoal, 6-14 mesh, activated at 200°C for 48 hours.
- 4.10. Carbon disulphide ( $CS_2$ ), spectroquality.
- 4.11. Glass wool.
- 4.12. **Calibration Standards**

- 4.12.1. Prepare calibration VCM standards containing hydrocarbons of similar retention times since these gases are commonly present in

ambient air. Calibration with these additional standards prevents misinterpretation of the GC peaks. Standards are prepared by serial dilutions of appropriate known volumes of hydrocarbons with nitrogen or clean air in either gas sampling bags or tubes.

**NOTE:** Gas-tight syringes can be used for sample injection into the GC to calibrate either gas sampling tubes or bags. However, if a gas sampling loop is used for injection, standards from gas sampling bags must be used.

#### 4.12.2. Standards in Gas Sampling Tubes

4.12.2.1. Insert about 10 small glass beads (Figure 2) into a 500 ml dilution vessel equipped with septum and 2 sidearms with stopcocks. Purge vessel and fill with purified air.

4.12.2.2. Using a 100  $\mu$ l syringe withdraw a known small quantity of VCM from a vinyl chloride gas cylinder (lecture bottle) fitted with a stainless steel adaptor for syringe sampling.

4.12.2.3. Inject gas into dilution vessel and mix with air by shaking for a period of not less than 10 minutes. Withdraw a similar size sample from this vessel and inject into another dilution vessel.

4.12.2.4. Continue this process until a concentration of VCM in air is obtained which is suitable for calibration purposes (usually between 0.03 and 10 ppm v/v).

**NOTE:** Glass vessels or sampling bags which have been used for the preparation of high concentrations should not be used for the preparation of low concentrations or for gas sampling unless thoroughly cleaned and tested.

#### 4.12.3. Standards for Gas Samples (Also used for Water, Soil and Vegetation Samples)

4.12.3.1. Inspect and flush a clean, deflated gas sampling bag and test as in 5.1.1. Partially fill with a known volume of nitrogen or clean air and seal (eg. 20 liters of air in 22 liter bag). A wet-test gas meter and a cartridge containing a drying agent can be used to meter and dry the clean air. Test a sample of clean air by GC to ensure that the metered air is not contaminated.

4.12.3.2. Inject pre-determined volumes (1 ml into 5 liters) of VCM (and other hydrocarbons) making up the standard through the septum of the bag using a gas-tight syringe. After all injections are completed gently knead the bag for a few minutes to mix the gases.

4.12.3.3. By serial dilution (as described in 4.12.2.3.) prepare working standards of 2 mg/l or less in sampling bags containing air or nitrogen. Use working standards within 24 hours of preparation.

**NOTE:** Unless thoroughly cleaned and tested, gas sampling bags used to prepare and store the high concentration standards should not be used to prepare working standards or to collect gas samples.

#### 4.12.4. Standards in Gas Adsorption Tubes

4.12.4.1. Using a gas-tight syringe, withdraw a known small quantity of the gas mixture from a dilution vessel prepared as in 4.12.2.1. and 4.12.2.2. Inject the gas mixture into the head of a sampling tube of activated charcoal through which air flows at a pre-determined flow rate. Inject varying concentrations of VCM in air into different charcoal tubes under otherwise identical conditions so that a calibration curve may be obtained.

### 5. Procedure

#### 5.1. Cleaning and Testing Air Sampling Bags and Tubes

- 5.1.1. Visually inspect gas sampling bags for damage then flush 3 times with clean air or nitrogen. Fill with clean air or nitrogen and let stand for 12 hours, then test by GC for contamination. Repeat flushing and testing as necessary. Bags satisfying GC test are deflated, sealed and used for sample collection or standard preparation. (Bags used for the latter purpose should not be reused for sample collection. Bags used for sampling heavily polluted air should be discarded.)
- 5.1.2. Before initial use, thoroughly wash gas sampling tubes with detergent solution, rinse first with distilled water and finally with methylene chloride. Purge clean tubes with clean air or nitrogen to remove traces of methylene chloride. Fill with clean air or nitrogen and test by GC for contamination.

#### 5.2. Air Bag Sampling (Instantaneous Grab Sample)

- 5.2.1. Use small sampling bags to obtain samples which are representative of vinyl chloride concentrations over sampling periods of up to a few minutes concentrations and methyl chloride.
  - 5.2.2. A gas sampling apparatus is shown in Figure 1. An inlet line filter for airborne particulate is optional. At the sampling site connect bubble tube and pump using a Teflon tube insert and activate pump. After sufficiently flushing pump and bubble tube with ambient air, insert tube into snout of deflated gas sampling bag.
  - 5.2.3. Collect sample, remove bubble tube from snout and seal bag by folding snout several times and securing it with a clip.
- 5.3. Grab samples can also be taken with a gas sampling train incorporating a gas sampling tube and pump as shown in Figure 2. Use a pump of sufficient capacity to flush tube and obtain a sample within a specified sampling period. At sampling site, activate pump, open both stopcocks and flush tube with at least 3 volumes of the air to be sampled. In rapid succession, close stopcock closest to the pump and then close the other stopcock.

#### 5.4. Integrated Sampling with Sampling Bags

- 5.4.1. Choose bag capacity (10-40 liters) according to sampling period and pump rate selected.
- 5.4.2. Gas sampling apparatus is shown in Figure 3. Set air flow prior to connecting bag. Restrict flow with an orifice (capillary or hypodermic needle) and adjust with bypass valve.
- 5.4.3. Flush train with air to be sampled and attach deflated bag to train. Use a timer to control length of sampling period. After sampling, remove bubble tube from snout and seal bag by folding snout several times and securing with a clip.

#### 5.5. Integrated Sampling With Gas Adsorption Tubes

- 5.5.1. Fill gas adsorption tubes with approximately 6 g activated charcoal in 2 equal segments separated by a 10 mm long glass wool plug in the centre. Place glass wool plugs at both ends of tube to prevent loss of charcoal and cover both ends with Teflon caps. Connect one end of the tube to calibrated air pump set at desired flow rate to provide a steady flow of air through the sampling tube. Sampling time is normally 30 minutes or more.

#### 5.6. Desorption from Gas Adsorption Tubes

- 5.6.1. Cool 2 glass stoppered test tubes containing carbon disulphide in an ice bath. Pour segments of exposed charcoal into each tube and immediately cap with glass stoppers.  
**NOTE:** Due to its toxicity and explosive nature, exercise caution when handling carbon disulphide.
- 5.6.2. Desorb the vinyl chloride from charcoal over a 90 minute period, frequently shaking the test tubes in the ice bath. Analyze the resulting carbon disulphide solutions separately by gas chromatography. (See 5.7. -5.9.)

#### 5.7. Water, Soil and Vegetation Samples

- 5.7.1. An aliquot of water or fresh undried soil or vegetation is placed in a hypo-vial, capped, and heated at about 85°C on a water bath for 2 hours.
- 5.7.2. A syringe is inserted through the membrane on the cap of the vial and a sample of the head space is taken and injected into the gas chromatograph. (See 5.8. to 5.9.)

#### 5.8. Operational Conditions of Gas Chromatograph

Column temperature: 50°C isothermal for 6 minutes, followed by temperature programming to 190°C at 16°C/min.

Detector temperature: 190°C



Injector temperature: 125°C

Nitrogen carrier gas flow rate: 20 ml/min.

Hydrogen gas flow rate: as required for optimum operation

Clean air flow rate: as required for optimum operation

Sample size: 1-5 ml gaseous sample or 1-5 µl CS<sub>2</sub> solution

#### 5.9. Calibration of the Gas Chromatograph

- 5.9.1. Adjust instrument to an appropriate sensitivity and stabilize to obtain a minimum noise level and a maximum zero-line stability.
- 5.9.2. For calibration using gas-tight syringes, inject a 1-5 ml volume of calibration standard into the GC and measure and record the peak height.
- 5.9.3. For calibration using a gas sampling loop, connect sampling bag to the sampling loop, flush loop and fill with standard. Inject gas in the loop into the GC and measure and record the peak height.
- 5.9.4. For calibration using gas adsorption tubes, desorb standards and using a syringe, inject 1-5 µl volumes of carbon disulphide solutions into the GC and measure and record the peak heights.
- 5.9.5. Prepare a calibration curve by plotting values obtained versus the amounts of VCM used. Calibrate GC daily when performing analyses.

#### 5.10. Gas Chromatographic Procedure

- 5.10.1. Isothermal screening is first performed using the following operating conditions:

Column temperature: 110°C, isothermal for 6 minutes

All other conditions are the same as those in 5.6.

- 5.10.2. Vinyl chloride and closely related other compounds are eluted from the column in the order shown in Figure 4.
- 5.10.3. If the screening procedure suggests the presence of vinyl chloride, adjust gas chromatograph to column temperature given in 5.8.
- 5.10.4. Under these conditions hydrocarbons are eluted from the column in the order shown in Figure 4. The retention time for vinyl chloride is approximately 13 minutes.
- 5.10.5. Completely remove any hydrocarbons from the column before analyzing the next sample. This may be achieved by one of the following procedures:
  - 5.10.5.1. Clear the column by maintaining the temperature at 190°C.
  - 5.10.5.2. Clear the column after the retention time for VCM by raising the temperature at the maximum available rate to 190°C.



5.10.5.3. Backflush column after the retention time for VCM by raising the temperature at the maximum available rate to 190°C.

## 6. Calculation and Reporting

- 6.1. For samples obtained from sampling bags and from the head space of water, soil and vegetation samples calculate the VCM concentration from its gas chromatographic peak as follows:

$$c = p \times F$$

Where:

- c = concentration of VCM in air in ppm.  
 p = peak height (mm).  
 F = response factor (ratio of working standard concentration to the GC peak height of the working standard, in ppm/mm).

- 6.2. For samples obtained from gas adsorption tubes, calculate the VCM concentration in ambient air as follows:

$$c = \frac{(a + b) \times F \times d}{e \times l}$$

Where:

- c = concentration of VCM in ambient air (ppm).  
 a = peak height for VCM from first charcoal segment (mm).  
 b = peak height for VCM from second charcoal segment (mm).  
 F = response factor  $\mu\text{l/mm}$  VCM (determined in 6.3).  
 d = volume of carbon disulphide used in desorption (20,000  $\mu\text{l}$ ).  
 e = volume of carbon disulphide solution injected into the GC (1-5  $\mu\text{l}$ ).  
 l = total air flow through gas adsorption tube (liters).

- 6.3. Obtain the average response factor (F) from the individual response factors (Fi) at certain concentrations of VCM as shown in the following example:

A VCM-air mixture containing 0.50  $\mu\text{l}$  of VCM is injected into a charcoal tube and the VCM desorbed with 20 ml carbon disulphide. A 3  $\mu\text{l}$  aliquot sample of this solution is injected into the gas chromatograph resulting in a VCM response with a peak height of 5 cm.

$$\begin{aligned} F_i &= \frac{\text{VCM } (\mu\text{l})}{\text{peak height}} \\ &= \frac{0.50 \times 3}{20 \times 1000} \times \frac{1}{50} \\ &= 1.5 \times 10^{-6} \mu\text{l/mm} \end{aligned}$$

Establish the Fi's at varying VCM concentrations and calculate the average F over the corresponding concentration range.

## 7. Precision and Accuracy

Results of replicate analyses of vinyl chloride in air should not differ by more than 10% of the mean. Accuracy has not yet been determined. The presence or absence of the compounds in the sample is more important than the actual concentration of the compounds detected.

## 8. Bibliography

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- 8.3. Levine, S. P., Hebel, K. G., Bolton Jr., J. and Kugel, R. E. (1975). Industrial Analytical Chemists and OSHA Regulations for Vinyl Chloride. Analytical Chemistry (12): 1075
- 8.4. Ott, M. G., Langner, R. R., and Holden, B. H. (1975). Vinyl Chloride Exposure in a Controlled Industrial Environment. Archives of Environmental Health: p. 333.
- 8.5. Purcell, J. E. (1975). Gas Chromatographic Analysis of Vinyl Chloride. American Laboratory, p. 99.

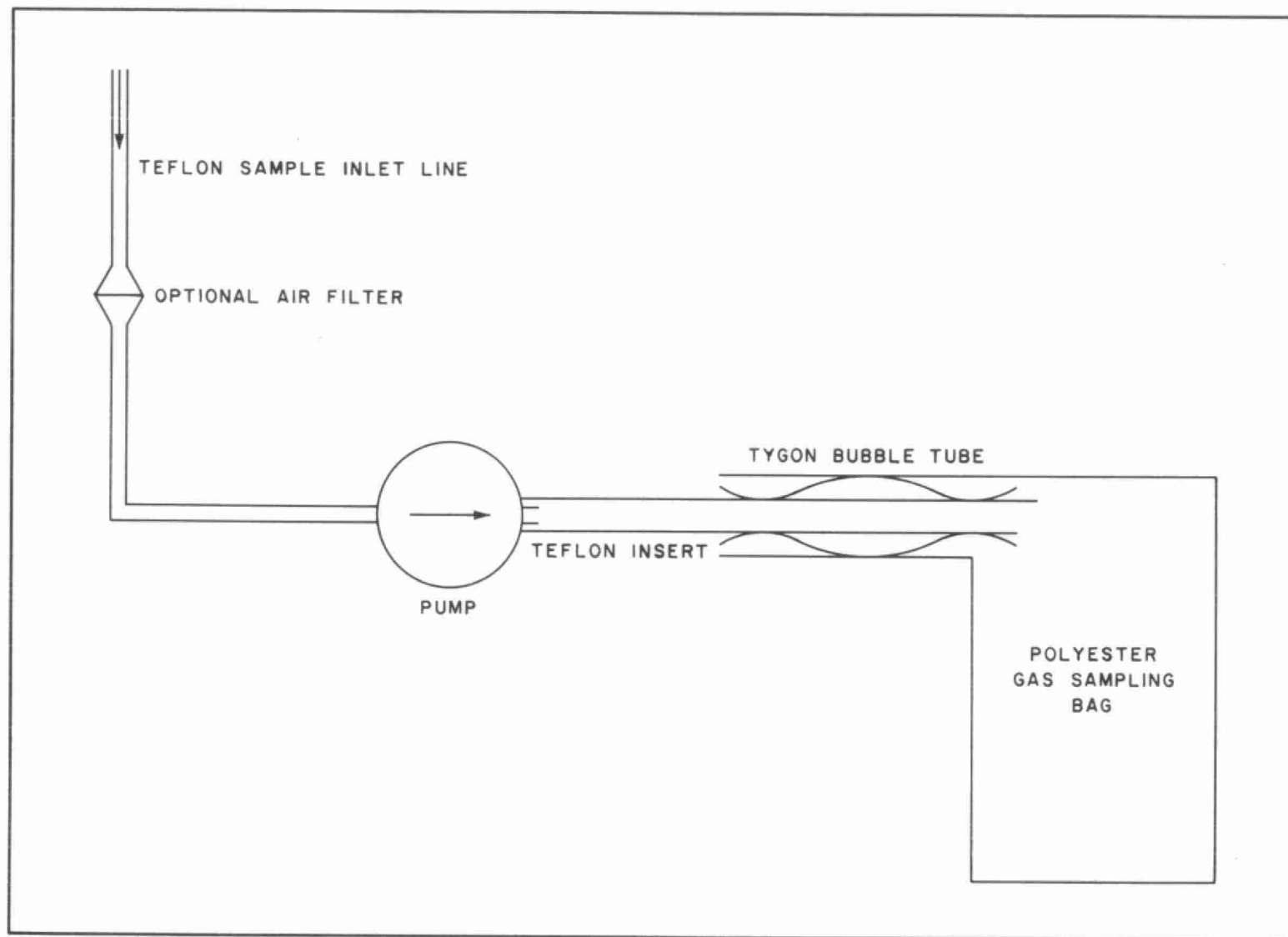


FIGURE 1 — GAS SAMPLING TRAIN (BAG FOR GRAB SAMPLING)

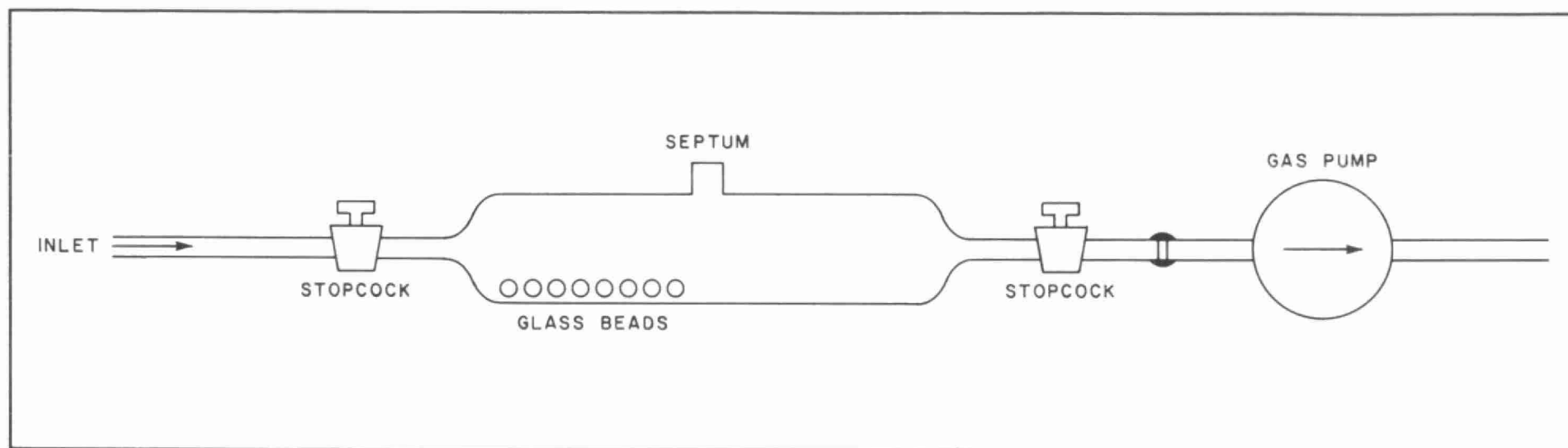


FIGURE 2 — GAS SAMPLING TRAIN-TUBE FOR GRAB SAMPLING OR FOR DILUTION OF CALIBRATION GASES

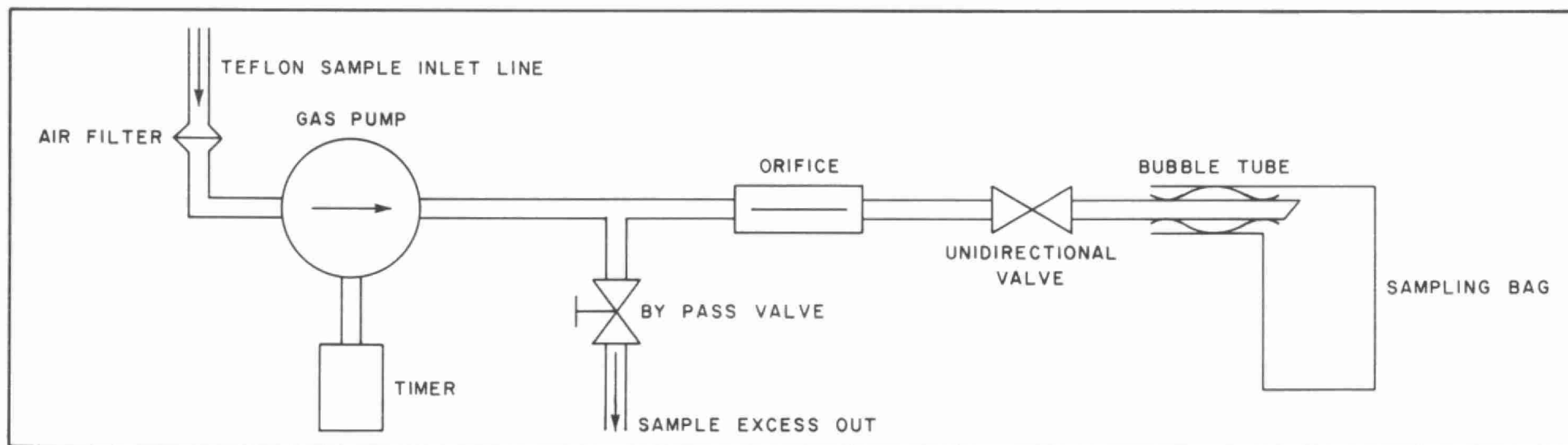


FIGURE 3 — GAS SAMPLING TRAIN-BAG FOR INTEGRATED SAMPLE

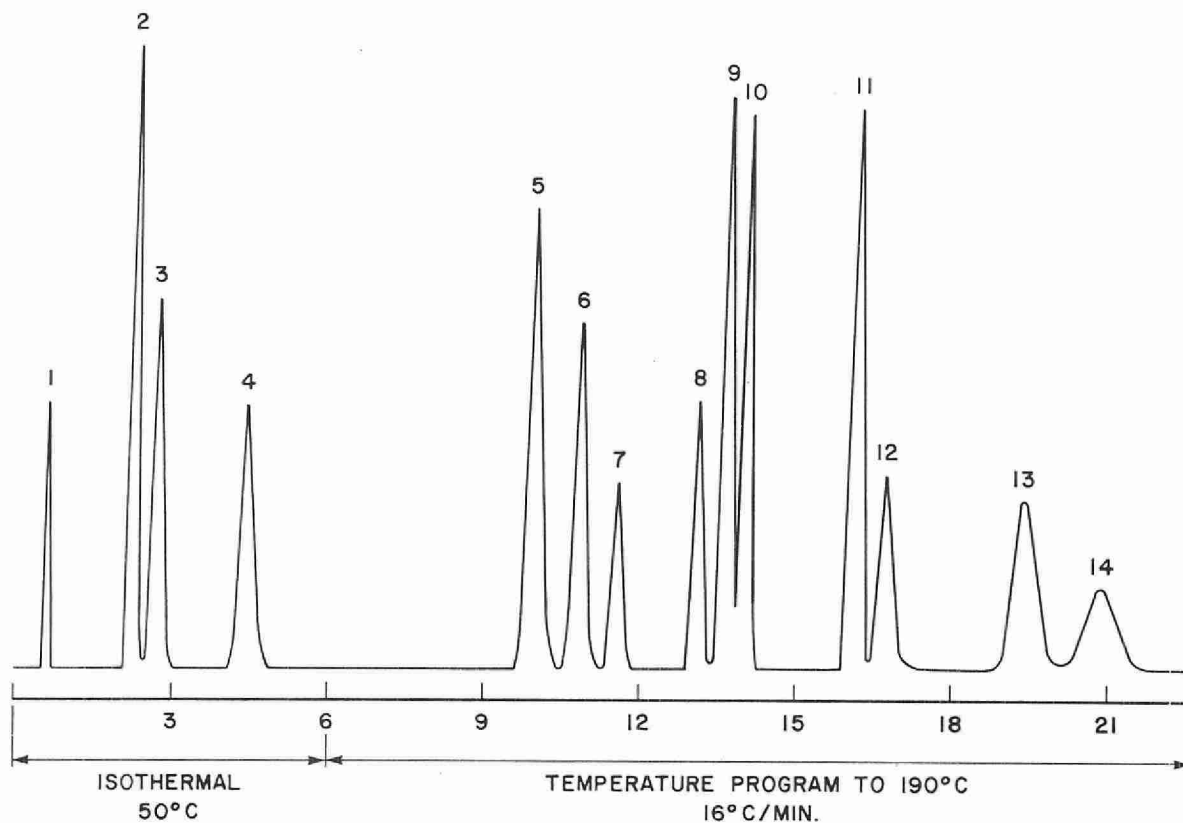


FIGURE 4 - SAMPLE GAS CHROMATOGRAM OF AIR CONTAINING:

- |                        |                           |
|------------------------|---------------------------|
| 1. METHANE             | 8. VINYL CHLORIDE         |
| 2. ETHYLENE            | 9. n-BUTENE + ISOBUTYLENE |
| 3. ETHANE              | 10. n-BUTANE              |
| 4. ACETYLENE           | 11. n-PENTANE             |
| 5. PROPANE + PROPYLENE | 12. METHYLENE CHLORIDE    |
| 6. CYCLOPROPANE        | 13. n-HEXANE              |
| 7. METHYL CHLORIDE     | 14. CYCLOHEXANE           |

## THE DETERMINATION OF VOLATILE ACIDS

The anaerobic degradation of carbohydrates, proteins and fats by saprophytic bacteria produces by-products such as volatile fatty acids, ammonia, alcohols, hydrogen sulphide, methane and carbon dioxide. Volatile fatty acids are water soluble short chain structures of low molecular weight and include acetic, propanoic, butyric and to a lesser extent valeric, isovaleric and caproic acids. These fatty acids are termed "volatile acids" because they can be distilled at atmospheric pressure.

Volatile acid data is useful in interpreting the performance of anaerobic digesters at sewage treatment plants.

### Sample Handling and Preservation

Samples should be collected in wide-mouthed jars, three-quarters filled and should be refrigerated prior to analysis.

### Selection of Method

The laboratory currently uses the column partition chromatography method for the determination of volatile acids. Gas chromatographic techniques are available which are capable of detecting individual acid components at extremely low concentrations; however, these techniques are unsuitable for routine analysis.

## VOLATILE ACIDS

### Column Partition Chromatography Method A

#### SUMMARY

<b>Matrix.</b>	This method is used routinely on sewage sludges.
<b>Substance determined.</b>	Volatile acids expressed as acetic acid.
<b>Interpretation of results.</b>	Results are expressed in mg/l acetic acid. Volatile acids are short chained organic acids; the test procedure does not differentiate among the chemical species. In an efficient anaerobic digestion system, the concentration level of volatile acids is relatively constant for any given treatment plant; a sharp increase or upward drift in the volatile acid concentration warns the operator of impending trouble.
<b>Principle of method.</b>	The sludge is centrifuged; an acidified aliquot of the supernatant is adsorbed on a column of granular inert silicic acid. A chloroform/n-butanol solvent is passed through the column to elute the organic acids. The eluate is collected and titrated with standard alcoholic 0.02N sodium hydroxide to the phenolphthalein endpoint.
<b>Time required for analysis.</b>	A single analysis requires 30 minutes. A batch of seven samples can be analyzed in two hours.
<b>Range of application.</b>	13 - 5000 mg/l acetic acid based on a 5.0 ml sample aliquot.
<b>Standard deviation.</b>	Based on within-run duplicates in the 13 - 5000 mg/l acetic acid range, standard deviations are: 7.63 for 0 - 20% of the range and 8.51 for 20 - 50% of the range.
<b>Accuracy.</b>	Accuracy is monitored by a 1000 mg/l quality control solution prepared with acetic acid. Average recovery is 103%, and the measured recovery does not vary by more than 5%.
<b>Detection criteria.</b>	12.55 mg/l acetic acid.
<b>Interferences and shortcomings.</b>	The chloroform/n-butanol solvent system is capable of eluting organic acids other than volatile acids and some synthetic detergents. Fortunately, the concentrations of these non-volatile components are insignificant.

**Minimum volume of sample.**

Sufficient sample to provide 10 ml of centrifuged supernatant.

**Preservation and sample container.**

Samples should be collected in wide-mouth jars, three-quarters filled, and refrigerated.

**Safety considerations.**

Safety glasses and gloves should be worn while handling acids and bases.

The chloroform/n-butanol reagent should be prepared and used in a fumehood only; care must be taken to avoid inhaling the vapour. When mixing the chloroform/n-butanol in the separating funnel, the gas build-up in the funnel should be vented from time to time.

Allow the solvent to vaporize from the used silicic acid in the fumehood before discarding.



## VOLATILE ACIDS

### Column Partition Chromatography Method A

#### 1. Introduction

After centrifuging the sample, an acidified aliquot of supernatant containing short-chain organic acids is adsorbed on a column of granular, inert, silicic acid. A chloroform/n-butanol solvent is passed through the column to elute the organic acids. The eluate is collected and titrated with standard 0.02N alcoholic sodium hydroxide to the phenolphthalein endpoint. The exact normality of the titrant is determined by standardization against a 0.02N potassium biphthalate solution to the phenolphthalein endpoint.

#### 2. Interferences and Shortcomings

Positive interferences may arise since the chloroform/n-butanol solvent is capable of eluting other organic acids (crotonic, adipic, pyruvic, phthalic, fumaric, lactic, succinic, malonic, gallic, aconitic and oxalic) as well as some synthetic detergents. However, these components are usually present in very low concentrations.

Atmospheric carbon dioxide may affect the titration endpoint extensively if a sample is subjected to excessive agitation. Extreme care must be taken to avoid vigorous mixing of the sample during titration.

#### 3. Apparatus

- 3.1. Centrifuge or filtering apparatus.
- 3.2. Crucibles, Gooch or fritted glass.
- 3.3. Separatory funnel, 1000 ml.
- 3.4. Pipet, volumetric, 5.0 ml.
- 3.5. Flask, Erlenmeyer, 250 ml.
- 3.6. Crucible adaptors, Gooch or fritted glass.
- 3.7. Buret, 10.0 ml with 0.05 ml divisions.

#### 4. Reagents

- 4.1. Silicic acid, ( $\text{SiO}_2 \cdot n\text{H}_2\text{O}$ ), 50 - 200 mesh.
- 4.2. Chloroform, ( $\text{CHCl}_3$ ), reagent grade.

- 4.3. n-Butanol, ( $\text{CH}_3\text{CH}_2\text{CH}_2\text{CH}_2\text{OH}$ ), reagent grade.
- 4.4. Sulphuric acid, ( $\text{H}_2\text{SO}_4$ ), concentrated, reagent grade.
- 4.5. Thymol blue indicator.
- 4.6. Phenolphthalein indicator.
- 4.7. Methanol, ( $\text{CH}_3\text{OH}$ ), reagent grade.
- 4.8. Sodium hydroxide, ( $\text{NaOH}$ ), reagent grade.
- 4.9. Potassium biphthalate ( $\text{COOH.C}_6\text{H}_4\text{.COOK}$ ), reagent grade crystals.
- 4.10 Acetic acid, ( $\text{CH}_3\text{COOH}$ ), glacial, reagent grade.

**4.11. Chloroform/n-Butanol Reagent**

Mix 300 ml reagent grade chloroform, 100 ml n-butanol and 80 ml 0.5N sulphuric acid in a separatory funnel. Drain off the lower organic layer through a fluted filter paper into a dry amber reagent bottle.

**4.12. Thymol Blue Indicator Solution**

Dissolve 80 mg thymol blue in 100 ml absolute methanol.

**4.13. Phenolphthalein Indicator Solution**

Dissolve 80 mg phenolphthalein in 100 ml absolute methanol.

**4.14. Stock Standard Sodium Hydroxide (1.0N)**

Dissolve 40 g NaOH in distilled, deionized water and dilute to 1000 ml. Extreme caution must be exercised in handling sodium hydroxide, a very caustic chemical.

**4.15. Standard Sodium Hydroxide (0.02N)**

Dilute 20 ml of 1.0N NaOH stock solution to 1 liter with absolute methanol. Standardize this working solution against potassium biphthalate.

**4.16. Q.C. Acetic Acid Solution (1000 mg/l)**

Weight out sufficient glacial Acetic acid so as to obtain 1.000 g Acetic acid and dilute to 1000 ml with distilled, deionized water.

**4.17. Sulphuric Acid Solution (0.5N)**

Slowly add 28 ml of concentrated sulphuric acid to 1.5 liter of distilled, deionized water and mix. Dilute to 2 liters with distilled, deionized water.

**4.18. Potassium Hydrogen Phthalate Solution (0.02N)**

Dissolve 4.085 g anhydrous potassium hydrogen phthalate (dried 2 hours at  $120^\circ\text{C}$ ) in 1 liter of carbon dioxide free distilled water.

### Standardization

Pipet 5.0 ml of standard potassium hydrogen phthalate solution (0.02N) into a 200 ml beaker. Dilute to 150 ml with distilled water. With continuous stirring, titrate the standard with the sodium hydroxide titrant to a pH of 8.3 or until a pink color persists for 30 seconds (use three drops of indicator).

## 5. Procedure

5.1. Centrifuge or vacuum-filter enough sludge to obtain 10 - 15 ml of clear supernatant in a small beaker.

5.2. Add 4 drops of thymol blue indicator solution, then concentrated sulphuric acid dropwise, until the color changes from red to thymol blue (pH 1.0 - 1.2).

### 5.3. Column Chromatography

5.3.1. Place approximately 12 g silicic acid in a fritted glass crucible.

5.3.2. Apply suction to pack the column.

5.3.3. With a 5.0 ml volumetric pipet, distribute the acidified sample evenly over the surface of the column.

5.3.4. Decrease the vacuum as soon as the last portion of the sample has entered the column.

5.3.5. Quickly add 65 ml chloroform/n-butanol reagent over the surface of the column, making sure that the silicic acid is kept under the solvent throughout the process.

5.3.6. Discontinue the suction just before the last portion of the chloroform/n-butanol reagent enters the column.

### 5.4. Titration

5.4.1. Remove the filter flask from the unit.

5.4.2. Titrate the contents with standard 0.02N sodium hydroxide to a phenolphthalein endpoint which persists for at least 30 seconds. Avoid aeration of the sample.

### 5.5. Blank

To approximately 50 ml distilled water add four drops of thymol blue indicator, then concentrated sulphuric acid until color change from red to thymol blue. Analyze 5.0 ml of this solution (steps 5.3 and 5.4).

## 6. Calculation and Reporting

- 6.1. Calculate the normality of sodium hydroxide as follows:

$$N_{\text{NaOH}} = \frac{N_{\text{KHP}} \cdot V_{\text{KHP}}}{V_{\text{NaOH}}}$$

Where:

$N_{\text{KHP}}$ : normality of potassium hydrogen phthalate

$V_{\text{KHP}}$ : volume of potassium hydrogen phthalate (5.0 ml)

$V_{\text{NaOH}}$ : volume of NaOH titrant used

- 6.2. Calculate total volatile acids (acetic acid) as follows:

$$\text{mg/l acetic acid} = \frac{(a - b)N \times 60 \times 1000}{5}$$

Where:

a = ml sodium hydroxide used for sample

b = ml sodium hydroxide used for blank

N = normality of sodium hydroxide

Report results to the nearest 5 mg/l.

## 7. Precision and Accuracy

Based on within-run duplicate samples in the 13 - 5000 mg/l acetic acid range, standard deviations are: 7.63 for 0 - 20% of the range and 8.51 for 20 - 50% of the range.

Accuracy is monitored by a 1000 mg/l quality control solution prepared with acetic acid. Average recovery is 103%, and the measured recovery does not vary by more than 5%.

## 8. Bibliography

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## THE ENUMERATION OF 35°C HETEROTROPHIC BACTERIA STANDARD PLATE COUNT

The enumeration of indicator bacteria serves the purpose of determining the sanitary quality of a water supply for consumption. The absence of indicator bacteria does not however signify that other bacteria are absent from the water. In fact, depending on the nutrient content of the water, the age and condition of the distribution system, the type of water treatment and the amount of chlorine residual in the water, general bacterial populations may range from only a few bacteria per ml to many thousands per ml. High "total" bacterial populations in the water of a municipal distribution system may adversely affect the overall water quality by contributing to taste and odour problems, enhancing conditions to cause biological fouling and persistence of pathogens, such as Pseudomonas aeruginosa and Staphylococcus aureus, and creating difficulties in maintaining any sort of chlorine residual at the outer extremities of the distribution system. Recommendations have been made towards applying an upper limit of 500 bacteria/ml on drinking water samples based on a geometric mean of five samples (8.1).

### Sample Handling and Preservation

Bacteriological samples should be collected in Ministry of the Environment (MOE) sterilized 250 ml glass bottles. The sample bottles should be chilled on ice and transported to the laboratory, where analysis should commence within 24 hours of the sampling time. Samples containing chlorine as a disinfectant should be collected in sterile sample bottles to which sodium thiosulphate has been added to neutralize any chlorine present. Usually the same bottles used for indicator organism analysis may be used for the Standard Plate Count analysis.

### Selection of Method

Standard Plate Count analyses have chiefly been performed in past years by a pour plate procedure (8.2). This particular procedure has a number of limitations which include a restriction on the volume of sample which can be analyzed to usually 1 ml or less; the need to keep the nutrient medium melted at 44-45°C before use; and frequent problems in counting because of spreading colonies at the interface of the medium and the petri dish. Although alternative methodologies have been tried for conducting analyses for "total bacterial counts", such as spread plates or spot plates, the most satisfactory method to date for analyzing drinking water samples on a routine basis is a membrane filtration test using a medium which was recently developed (8.3).

## 35°C HETEROTROPHIC BACTERIA MEMBRANE FILTER ANALYSIS

### SUMMARY

Matrix	This method is used routinely on drinking water samples.
Organisms determined	The test is designed to measure the number of heterotrophic bacteria which may develop on a nutrient rich medium at 35°C from distribution system water samples.
Interpretation of results	Bacterial counts in excess of 500 organisms per ml based on a geometric mean of five samples indicate poor water quality.
Principle of method	Each bacterial cell deposited on the membrane filter and given a suitable nutrient source plus the ability to grow and multiply at 35°C has the potential to develop into a bacterial colony.
Time required for analysis	The analysis time for one sample is approximately 2 minutes. The incubation time is $48 \pm 2$ hours, although both 24 and 48 hour counts may be useful for interpretive purposes.
Range of application	The analytical volume used for routine drinking water samples is 1 ml, however sample volumes of 100 ml or less may be conveniently filtered.
Standard deviation	Performance characteristics are not yet available.
Accuracy	Performance characteristics are not yet available.
Detection criteria	Performance characteristics are not yet available.
Interferences and shortcomings	Very poor quality water may have bacterial numbers too high to be counted accurately when the 1 ml sample volume is filtered routinely and dilutions may be required to get a definitive count. Spreading colonies may occasionally obscure other bacterial colonies on the filter and result in only an estimated count.
Minimum volume of sample	A minimum of 200 ml of sample is preferred for performing the SPC analysis and other corresponding analyses for indicator organisms.
Preservation and sample container	MOE 250 ml sterile glass bottles should be used for collection of samples. Samples should preferably be iced or refrigerated from the time of sampling to the time of analysis and should arrive at the laboratory within 24 hours. Water samples containing chlorine should be collected in sample bottles containing sodium thiosulfate.

**Safety  
considerations**

Bacteriological samples, particularly those from sources of suspected pollution, may contain pathogens and should be handled in a manner to prevent contamination of the analyst and the sampler.

## **35°C HETEROTROPHIC BACTERIA MEMBRANE FILTER PROCEDURE**

### **1. Introduction**

The Standard Plate Count analysis by membrane filtration employs a modification of a medium by Taylor and Geldreich (8.3). The modification consists of the addition of 0.02g/l of Bromocresol Purple to give colour to the bacterial colonies and assist with the recognition of minute colonies as well as relieve the tedium of counting white colonies on a white membrane filter. Although bacterial counts will vary depending on the water quality and incubation temperature, a standard sample volume of 1 ml and an incubation temperature of 35°C will provide information to best assess the water quality in terms of those bacteria which may affect human health after consumption.

### **2. Interferences and Shortcomings**

The enumeration of bacterial colonies using the standard sample volume of 1 ml may in some cases result in no observable colonies and in other instances the number of bacteria per 1 ml may be too excessive to be counted accurately. At other times, spreading colonies or fungal growth may obscure some or all of the filter and interfere with enumeration.

Unless the bacteria have the ability to grow and multiply on a nutrient-rich medium at 35°C within 48 hours, some organisms may not develop sufficiently to form bacterial colonies.

### **3. Apparatus**

#### **3.1. Sterile Equipment**

- 3.1.1. Pipettes, 1 ml
- 3.1.2. Membrane filters with grid (0.45 µm pore size), 47 mm
- 3.1.3. Membrane filter unit consisting of a base and funnel stored within a sterile container.

#### **3.2. Non-Sterile Equipment**

- 3.2.1. Beaker, 50 ml, containing 20 ml of 95% ethanol for flaming forceps
- 3.2.2. Blunt-end forceps for handling membrane filters
- 3.2.3. Polyethylene jar (2 liter) for used pipettes
- 3.2.4. 2 outlet vacuum Erlenmeyer, 2 liter (waste collection) flask with vacuum tubing and one-way valve connected to the bottom outlet.



- 3.2.5. Vacuum Erlenmeyer 1 liter, 2 outlet, plugged, (water trap) flask connected with vacuum tubing, from the bottom outlet to the 2 liter flask and from the top outlet to the vacuum source.
- 3.2.6. Polyethylene bucket for waste water
- 3.2.7. Bunsen burner
- 3.2.8. Plastic "cakettes" for incubation of petri dishes
- 3.2.9. Retort stand and clamp for holding membrane filter unit funnel when membrane filter is being removed from the base and placed on the bacteriological medium.

### 3.3. Accessory Equipment

- 3.3.1. Incubator for maintaining temperature at 35 °C
- 3.3.2. Stereoscopic microscope with 10X magnification
- 3.3.3. Cool white fluorescent light for illuminating bacterial colonies
- 3.3.4. Slanted (15 °) wooden stage for resting petri dish during the counting operation

## 4. Reagents

- 4.1. Sodium hydroxide solution (1 N)  
In a 1 liter volumetric flask dissolve 40.0 g sodium hydroxide in distilled water and dilute to the mark.
- 4.2. Potassium dihydrogen orthophosphate solution (0.25 N)  
In a 1 liter volumetric flask dissolve 34.0 g of potassium dihydrogen orthophosphate in distilled water and dilute to the mark.
- 4.3. Buffered Water  
To 1000 ml of distilled water add 1.25 ml of 0.25 M potassium dihydrogen phosphate (previously adjusted to pH 7.2 with 1 N sodium hydroxide). Prepare in a 4 l rinse bottle with dispensing nozzle and sterilize in an autoclave for 40 minutes at 121 C.
- 4.4. Disinfectant for swabbing benches, disinfecting pipettes, and filter effluent:
  - 4.2.1. Wescodyne ("tamed iodine" - 1:30 with water)
  - 4.2.2. Dettol (1:5 with water)
- 4.5. Bacteriological Medium: m-Standard Plate Count agar (m-SPC) modified with the addition of Bromocresol Purple and poured into 15 x 100 mm square petri dishes.

## 5. Procedure

### 5.1. m-Standard Plate Count Analysis

5.1.1. This test will normally be done along with other tests for indicator organisms, such as the total coliform and presence-absence analysis. After the control filter for each sample has been done, one ml of the sample is put through a membrane filter. The membrane filter is transferred to the m-SPC medium and placed on one quarter section of the 15 x 100 mm square petri dish. The three remaining quarter sections receive membrane filters from the next three samples respectively. The plates are placed in plastic cakettes along with a moistened paper towel for humidity. Incubation is at 35°C for 24 ± 2 hours to obtain an initial 24 hour count, followed by reincubation for an additional 24 ± 2 hours to obtain the 48 hour count.

5.1.2. The m-SPC plates are counted using the stereoscopic microscope at a 10X magnification. The plates are placed on the slanted stage for counting to achieve the best illumination for seeing the large as well as the small pin-point colonies. The ideal density of colonies on a membrane filter would consist of a total count not exceeding 300 colonies per ml. However, depending on the size of the colonies, higher counts may be made by taking the colony count in 5 to 10 of the grid squares and averaging the counts. The average count per square X 100 = the count per ml. Using this system, estimated counts may be made up to 2400 organisms per ml before difficulties are experienced in keeping track of the colonies in each square. Counts of this type will only be made when colonies are sufficiently small and discrete and do not merge or coalesce together.

## 6. Calculation and Reporting

The standard plate count for this laboratory is performed using membrane filtration procedures. This test is routinely done by using a sample volume of one ml with incubation at 35°C for 48 hours on the m-SPC medium. In some instances, 48 hour counts may be unsatisfactory because of excessive numbers of colonies on the membrane filter and 24 hour counts may be employed to assess the water quality (8.4.). For monitoring the overall water quality of a municipal distribution system, a geometric mean should be taken on the basis of the analysis of at least five samples. If the geometric mean exceeds 500 organisms per ml, the water quality is considered poor and corrective measures should be taken to improve the water quality (8.1.).

## 7. Preparation of the Medium for the Standard Plate Count (SPC) Analysis

### 7.1. Medium for "total bacterial counts" or 35°C Heterotrophic Bacterial Counts

#### 7.1.1 Apparatus Required for the Preparation of the m-SPC Medium

- 7.1.1.1. Thermometer (0-110 °C)
- 7.1.1.2. Stirring hot plates
- 7.1.1.3. Asbestos gloves
- 7.1.1.4. Top loading balance sensitive to 0.01g

- 7.1.1.5. Analytical balance sensitive to 0.0001g
- 7.1.1.6. Bunsen burner
- 7.1.1.7. Large and small spatula
- 7.1.1.8. Large stirring magnet
- 7.1.1.9. Graduated cylinder, one liter
- 7.1.1.10. Aluminum foil
- 7.1.1.11. Large forceps
- 7.1.1.12. Beaker (glass), two liter
- 7.1.1.13. Petri dishes, 15 x 100 mm square, sterile plastic (approx. 50-60)
- 7.1.1.14. Weighing boat and weighing paper
- 7.1.1.15. Small fan
- 7.1.1.16. Beaker, 600, ml sterile
- 7.1.1.17. 95% ethanol in 100 ml beaker
- 7.1.1.18. Plastic cakettes
- 7.1.1.19. pH meter

**7.1.2. Reagents Required for Preparation of 1 Liter of m-SPC Medium**

- |                             |         |
|-----------------------------|---------|
| 7.1.2.1. Gelatin            | 25.0g   |
| 7.1.2.2. Peptone            | 20.0g   |
| 7.1.2.3. Agar               | 15.0g   |
| 7.1.2.4. Glycerol           | 12.6g   |
| 7.1.2.5. Bromocresol Purple | 0.02g   |
| 7.1.2.6. Distilled Water    | 1000 ml |

**7.1.3. Preparation of the m-SPC Medium**

- 7.1.3.1. Check the balances to ensure that they are level and operational. Zero each balance.
- 7.1.3.2. Place the 2 l glass beaker on a stirring hot plate and using the graduate cylinder, measure out 1000 ml of distilled water and pour it into the beaker. Using the bunsen burner, flame the stirring magnet which was previously dipped in the 95% ethanol and drop the magnet into the 2 l beaker. Activate the stirring mechanism.

- 7.1.3.3. Using the large spatula, weigh out individually into the weighing boat, the gelatin and peptone. Pour separately into the 1000 ml of distilled water. Pour sufficient glycerol into the weighing boat until the correct weight is obtained and then dissolve in the distilled water with the other ingredients by gentle heating. Measure the pH of the entire volume of medium. Adjust to pH 7.1 with 1 N NaOH, if required. Weigh out the agar and add to mixture.
- 7.1.3.4. Using the small spatula and the analytical balance weigh out the Bromocresol purple onto a piece of weighing paper and dissolve the indicator with the other ingredients.
- 7.1.3.5. Place the 2 l beaker and contents on another stirring hot plate and activate both the stirring and heating components. Cover the beaker with aluminum foil, insert the thermometer, and heat the mixture to 90°C to dissolve the agar and other ingredients.
- 7.1.3.6. After the medium has reached 90°C, the thermometer is removed and the opening is sealed with autoclave tape. Using the asbestos gloves, transfer the 2 l beaker to the autoclave and sterilize the medium at 121°C for 15 minutes.
- 7.1.3.7. Following sterilization, the 2 l beaker is placed on a stirring device in front of a fan and allowed to cool to 60°C. A thermometer previously disinfected in alcohol may be used to monitor the cooling of the medium.
- 7.1.3.8. During this period, the petri plates are set out on the bench which has been previously swabbed with dettol.
- 7.1.3.9. When the medium has cooled sufficiently, about 500 ml are poured into the sterile 600 ml beaker for convenience in pouring the medium into the 15 x 100 mm square petri dishes. Flame thoroughly around the aluminum foil cover and edges of each beaker before and after transfer of the medium. Place the remainder of the medium in the 2 l beaker back on the stirring plate until needed.
- 7.1.3.10. About 20 ml of the medium is poured into each petri dish to give a medium depth of 3-4 mm. The lids of the petri dishes are left about one quarter open to allow cooling and solidification of the medium without excess condensation forming on the lids.
- 7.1.3.11. After about 20 minutes the medium should be thoroughly solidified and the petri dish lids should be closed; the petri dishes should then be stacked in an inverted position in cakettes; the cakettes are stored in a darkened area at room temperature for 24 hours to check for contamination before placing them in the refrigerator at 4°C. One of the plates should be removed beforehand to check on the pH which should be  $7.1 \pm 0.2$  at 25°C.

7.1.3.12. Each cakette is labelled with the date of preparation and the initials of the person who prepared the medium. The plates may be stored at refrigeration temperatures in the moisture tight containers for up to four weeks.

7.1.3.13. Clean up the preparation area including the weighing scales, stirring hot plates, and work benches. Wash the beakers in hot water to remove all traces of the agar medium.

7.1.3.14. A laminar flow unit if available should be used for pouring the medium into the petri dishes.

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## **PRESENCE-ABSENCE (P-A) PROCEDURE FOR DETECTION OF INDICATOR BACTERIA IN DRINKING WATER SUPPLIES**

The methodology for detection and enumeration of total coliforms, fecal coliforms and fecal streptococci by membrane filtration has been described elsewhere. In this section, a methodology is described for the detection of these organisms by the presence-absence (P-A) test. This test has proven more sensitive for detecting pollution indicator bacteria in drinking water samples than the membrane filter (MF) procedure. Essentially the P-A test is a modification of the most probable number (MPN) procedure, which uses a dilution series of broth tubes to detect and enumerate microorganisms. The P-A test employs only one analysis bottle per sample to determine a wide variety of pollution indicator bacteria.

### **Sample Handling and Preservation**

Bacteriological samples must be collected in Ministry of the Environment (MOE) sterilized glass bottles. The bacteriological examination of a water sample should be initiated immediately after collection. However, as this is seldom practical, more realistic arrangements must be established. The sample bottle should be transported to the laboratory in 24 hours. Bacteriological MF analyses are not done on samples aged 3 days or more. Samples containing chlorine as a disinfecting agent should be collected in sterile bottles to which sodium thiosulphate has been added to neutralize any chlorine present.

### **Selection of Method**

Municipalities require analysis of their raw or source water, as well as the water at various stages of the treatment process in the plant and also after the water has left the plant and entered the distribution system. The laboratory analyzes samples of these waters by either the presence-absence (P-A) or membrane filter (MF) technique, or, in some instances, a combination of both techniques:

- a) Raw water samples will be analyzed by the MF procedure for total coliforms (TC), fecal coliforms (FC), and fecal streptococci (FS), when TC, FC and FS counts are 10 or more per 100 ml based on previous analyses. If counts are less than 10 for any of the indicator organisms, a P-A test should be performed along with the MF test for total coliforms.
- b) Plant samples will usually be analyzed for total coliforms and background counts by the MF test unless the enumeration of other indicator organisms is deemed necessary or requested. A P-A test will be included as part of the analysis for these samples.
- c) Distribution samples will be initially examined by a combination of both MF and P-A tests. After establishing that these samples usually give negative results with the MF test, all samples will receive P-A tests and periodic random samples will be picked out and given both MF and P-A tests. This will

be done usually on a one in four to one in eight basis, for the purpose of checking on background counts as well as possible total coliform counts. Any sample locations previously having pollution indicator organisms detected by the P-A test will receive an MF/P-A test combination on their next submission. Samples consecutively positive by both MF and P-A tests may be analyzed by only MF tests on subsequent sample submissions.

## PRESENCE-ABSENCE TEST ANALYSIS

### SUMMARY

Matrix	This method is used on drinking water samples.
Organisms determined	The test is designed to qualitatively detect a variety of pollution indicator organisms in water, including total coliforms, fecal coliforms, fecal streptococci, <u>Pseudomonas aeruginosa</u> , <u>Staphylococcus aureus</u> , <u>Aeromonas</u> sp. and <u>Clostridium perfringens</u> .
Interpretation of results	The presence of any of the above groups represents inadequately treated and possibly hazardous water. For more detailed information, consult the MOE's Drinking Water Objectives.
Principle of methods	Each of the above pollution indicator organisms may be present alone or in combination with each other. A selection of confirmatory and taxonomic tests permits differentiation of the bacteria present.
Time required for analysis	The initial analysis time is less than one minute per sample. Time required for presumptive results ranges from one to five days. Time required for confirmatory results is from one to four days depending on the pollution indicator group present.
Range of application	Maximum sample volume for which an analysis is usually done is 100 ml; the minimum sample volume is usually 50 ml.
Standard deviation	Performance characteristics are not applicable.
Accuracy	Performance characteristics are not yet available.
Detection criteria	Performance characteristics are not yet available.
Interferences and shortcomings	Because the P-A test is qualitative in nature, samples giving a positive test for pollution will require a MF or MPN analysis to determine numbers of organisms. Analysis of sample volumes greater than 100 ml will also require either MF or MPN methods.
Minimum volume of sample	200 ml of sample is preferred particularly if both MF and P-A analyses are performed on the sample.



**Preservation and  
sample container**

MOE 250 ml sterile glass bottles should be used for collection of samples for drinking water analyses. Those for collection of chlorinated water samples must contain sodium thiosulphate to neutralize any chlorine present. Samples should arrive within 24 hours of the sampling time.

**Safety  
considerations**

Bacteriological samples, particularly those from sources of water suspected of pollution, may contain pathogenic organisms, and should be handled in a manner to prevent contamination of the sampler and the analyst.

## POLLUTION INDICATOR BACTERIA

### PRESENCE-ABSENCE ANALYSIS

#### 1. Introduction

The determination of total coliforms, fecal coliforms, fecal streptococci, Pseudomonas aeruginosa, Staphylococcus aureus, Aeromonas sp. and Clostridium perfringens bacteria by the presence-absence (P-A) test is performed by initially adding the water sample to a bottle containing the presumptive medium. Then, by a selection of confirmatory and taxonomic tests performed on a presumptive positive P-A bottle, any or all of the above groups of organisms may be isolated and identified. In some instances, total coliform and fecal coliform cultures may be isolated and identified at the generic level.

#### 2. Interferences and Shortcomings

The identification of the different types of organisms present in water samples, particularly those representative of pollution indicator organisms, depends to a certain extent on their initial numbers in the original sample and their ability to grow and multiply in the mixed population existing in the presumptive P-A bottle. Following transfer to confirmatory media, which usually contain substances that preferentially select out certain organisms for growth and inhibit others, the isolation of particular organisms often depends on the skill of the technician to streak out mixed cultures on agar plates in order to obtain isolated, individual colonies. Sometimes, the isolation of particular types of organisms is made difficult because of the overgrowth on bacterial agar plates by other organisms present in larger numbers.

Other factors influencing the isolation of representative types of bacteria from the original sample will depend on storage conditions during transit to the laboratory and whether the sample contains any nutritive or inhibitory substances.

#### 3. Apparatus

##### 3.1. Sterile Equipment

- 3.1.1. Pipettes - 1 ml

##### 3.2. Non-sterile Equipment

- 3.2.1. Incubation racks for 250 ml bottles
- 3.2.2. Bunsen burner
- 3.2.3. Inoculating loop(s) and needle(s)
- 3.2.4. Wax crayon and marking pen for numbering P-A bottles, test tubes and petri dishes
- 3.2.5. Test tube racks for holding 40 test tubes
- 3.2.6. Plastic "cakettes" for incubation of petri dishes

- 3.2.7. External measuring device for 100 ml volumes: 4" steel shelf bracket with one side cut off at 86 mm.

### 3.3. Accessory Equipment

- 3.3.1. Waterbath and/or incubators for maintaining temperatures within  $\pm 0.5^{\circ}\text{C}$  in the ambient to  $60^{\circ}\text{C}$  range.
- 3.3.2. Stereoscopic microscope with 10X magnification for examining bacterial colonies.
- 3.3.3. Cool white fluorescent light for illuminating bacterial colonies.
- 3.3.4. Slanted ( $15^{\circ}$ ) wooden stage for resting petri dish during the examination of bacterial colonies.
- 3.3.5. Daylight fluorescent titration illuminator for examining bacterial growth in P-A bottles, test tubes and agar plates. A magnifying device with a built-in illuminator may also be used for examining and isolating bacterial colonies.
- 3.3.6. Longwave ultraviolet (hand-held) light with wavelength of 366 nm.

## 4. Reagents

### 4.1. Buffered Water.

To 1000 ml of distilled water add 1.25 ml of 0.25 M  $\text{KH}_2\text{PO}_4$  (previously adjusted to pH 7.2 with 1N NaOH). (See "The Enumeration of Total Coliforms, Fecal Coliforms and Fecal Streptococci" for instructions on the preparation of these reagents). The buffered water is made up in dilution blanks and sterilized in an autoclave to result in a final volume of  $99 \pm 1$  ml.

### 4.2. Disinfectant for swabbing benches and disinfecting pipettes

- 4.2.1. Wescodyne (1:30 with water)
- 4.2.2. Dettol (1:5 with water)

### 4.3. Reagents for Gram stain - see 8.1. for preparation instructions

### 4.4. Bacteriological media:

- 4.4.1. P-A bottles - MacConkey broth, (presumptive medium)
- 4.4.2. P-A bottles-Lactose Lauryl Tryptose broth, (alternative presumptive medium)
- 4.4.3. EC broth
- 4.4.4. Drake's broth
- 4.4.5. Ethyl Violet Azide (EVA) broth
- 4.4.6. Skim Milk broth
- 4.4.7. Lactose Purple broth
- 4.4.8. Trypticase Soy broth
- 4.4.9. MacConkey agar plates
- 4.4.10. Enterococcus agar plates

- 4.4.11. Nutrient Gelatin agar plates
- 4.4.12. Skim Milk agar plates
- 4.4.13. Mannitol Salt agar plates

The instructions for preparation of the above media will be found in section 7.

## 5. Procedure

### 5.1. Presumptive Part of P-A Test

- 5.1.1. Samples should be analyzed the same day as received, otherwise they must be kept refrigerated before analysis.

- 5.1.2. Preparation for the presence-absence (P-A) test

- 5.1.2.1. Sterile technique must be employed throughout the analytical procedure.
  - 5.1.2.2. The work bench area is washed thoroughly with the Dettol solution and wiped dry.
  - 5.1.2.3. The samples are arranged on the work bench in numerical order according to the laboratory number.
  - 5.1.2.4. Each P-A bottle is marked with the laboratory number corresponding to that on the sample bottle.

- 5.1.3. Presumptive Presence-Absence (P-A) Analysis

- 5.1.3.1. The sample bottle is shaken vigorously, placed on the bench, and the cap loosened. The 86 mm side of the shelf bracket is placed up against the side of the P-A bottle. The cap of the sample bottle is removed and the mouth of the bottle is flamed. While holding the cap of the sample bottle in one hand, the cap of the P-A bottle is removed and held in the other hand; the mouth of the P-A bottle is also flamed.
  - 5.1.3.2. The sample bottle is picked up again and 100 ml of sample is poured out into the P-A bottle, using the top of the 86 mm side of the shelf bracket as a guide to indicate the proper volume; the mouth of the bottle is flamed; and the cap is screwed back into place.
  - 5.1.3.3. The P-A bottle is inverted four to five times to mix the sample and growth medium.
  - 5.1.3.4. Each P-A bottle is transferred to a rack and placed in the 35°C incubator for four to five days.

- 5.1.4. Incubation Period Observations (P-A Bottles)

- 5.1.4.1. Each day for four to five days (samples analyzed on Wednesday have their last incubation day on the following Monday, i.e. 5 days), the P-A bottles are removed from the incubator and examined for any colour change or gas production.

- 5.1.4.2. If pollution indicator organisms are present in the sample added to the P-A bottle, fermentation of the lactose sugar will occur. This results in acidic conditions being produced and these conditions are monitored by a bromocresol purple pH indicator in the broth. As the medium becomes acidic, the colour changes from purple to yellow and the intensity of the change is subjectively recorded by the letters:  
a, (A), and A respectively representing weak to strong acid conditions.
- 5.1.4.3. No change or no growth is recorded as O, and no colour change, but bacterial growth, is recorded as +.
- 5.1.4.4. If the lactose fermentation is also accompanied by gas production, gentle shaking of the P-A bottle several times will result in a foaming reaction. If foam only forms on the medium surface in contact with the sides of the bottle, the reaction is marked as f; if a foam layer covers the entire surface of the medium, the reaction is marked as F..
- 5.1.4.5. Any P-A bottle which has been scored as a, (A), A or f, F or any combination of these symbols is removed from the rack for the performance of confirmatory tests.
- 5.1.4.6. Any P-A bottle scored as O or + is left in the rack and the rack is returned to the 35°C incubator. These bottles are checked again every 24 hours until the end of their scheduled incubation period to determine whether further changes have taken place which would require confirmatory tests. If none of these tests is required on the bottles, they are discarded after four (or five) days.

**NOTE:** discarding of any bacterial analyses where growth of organisms has occurred, means that the discarded material is autoclaved before ultimately disposing of it.

## 5.2. Confirmatory Analyses of the P-A Test

- 5.2.1. P-A bottles with any combination of acid or acid and gas reactions receive the following tests:

EC broth (48 h)  
MacConkey agar plate (24 h)  
EC broth (44.5°C) (48 h)  
Enterococcus agar plate (48 h)  
Drake's tube (41.5°C - 72 h)  
Mannitol Salt Agar plate (48 h)  
Ethyl Violet Azide (EVA) tube (48 h)  
Skim Milk tube (48 h)

All of the above confirmatory tests are incubated at 35°C unless otherwise indicated. Depending on the results of the initial confirmatory tests, further confirmatory tests are usually employed with each indicator group to ensure that each group has been properly characterized (See Presence-Absence (P-A) Test Scheme).

5.2.2. Method of inoculation, incubation period, type of result and additional testing required for the above confirmatory tests.

5.2.2.1. All confirmatory media should be at room temperature before inoculation takes place and agar plates should be checked to insure the agar surface is dry before streaking with inoculum. Plates with moisture droplets can be placed in a 35°C incubator for 15-20 minutes or a laminar flow unit with the lids ajar to allow the air of the incubator or laminar flow unit to dry off the surface of the plate and minimize any contamination.

Confirmatory tube tests are grouped together in a test tube rack and agar plates are divided in half with a marking pen. All tubes and agar plates are prenumbered and organized usually in numerical order before any inoculations are begun.

5.2.2.2. The transfer of inoculum from a P-A bottle to test tube broths requires a number of steps. If bacterial growth in the P-A bottle has tended to settle to the bottom, slight agitation will redisperse the bacterial growth throughout the growth medium. If the bacterial growth is uniformly distributed throughout the growth medium, agitation will not likely be required. If the P-A bottle shows any gas production, the cap of the bottle should be loosened to relieve any pressure buildup and then retightened before any agitation of the medium takes place.

One or more 3-4 mm inoculating loops should be available to the technician. Just before use, the wire and loop portion of the inoculating loop should be heated in a bunsen burner flame until they are red hot and then allowed to cool. The cap of the P-A bottle is removed and the mouth of the P-A bottle is flamed. One of the sterilized inoculating loops is introduced into the mouth of the P-A bottle and lowered until the loop is submerged 10-20 mm below the surface of the medium. The loop is then withdrawn carefully from the P-A bottle (avoid touching mouth or sides of bottle) and the cap is placed back on the mouth of the P-A bottle. While the loop is being held in a horizontal position to avoid losing the inoculum contained in the loop, the test tube being inoculated is removed from the test tube rack and the metal cap is taken off. The mouth of the test tube is flamed and the inoculating loop is introduced into the mouth of the test tube and lowered until the loop is below the surface of the medium (avoid touching the sides of the test tube). The loop is dipped several times below the surface of the medium before withdrawing the loop from the test tube. The mouth of the test tube is flamed again; the cap is replaced; and the test tube is placed back in its rack again and slanted or turned in a manner to indicate that it has been inoculated. The loop is then flamed until it is red hot being careful to avoid any splattering of inoculum remaining on the loop. This procedure is repeated for all tubes being inoculated or if more than one loopful of inoculum is taken from the same P-A bottle.

- 5.2.2.3. The streaking of agar plates to obtain isolated colonies of bacteria requires the following procedure. A smaller inoculating loop (i.e. 2-3 mm) is preferred for streaking agar plates than the one used for inoculating broth tubes. The loop is sterilized in a bunsen burner flame just prior to use. The cap of the P-A bottle is removed aseptically; the inoculating loop is introduced into the P-A bottle; submerged in the broth medium; withdrawn and held in a horizontal position as previously described; and the cap is returned to the P-A bottle.

The bottom portion of the petri dish (containing the agar medium) is picked up with the thumb and fingers of the hand adjacent to the ends of the line drawn to divide the area of the petri dish in half. The petri dish is held at an angle of  $45^\circ$  to the vertical, while the inoculating loop streaks the inoculum on the agar surface. The inoculum is deposited on one half of the petri dish in three equal sections. Using a gentle sweeping motion and non-overlapping streaks, the major portion of the inoculum is deposited in the upper right hand section; the plate is turned  $90^\circ$  and the middle section of the upper half of the plate is streaked; the plate is turned  $90^\circ$  and the upper left hand (third) section of the plate is streaked. The petri dish bottom is returned to the lid and the entire plate is rotated  $180^\circ$  to put it in position to streak the second half of the plate with another culture. The inoculating loop is sterilized in the flame as before. This procedure is repeated for all plates being inoculated from P-A bottles.

- 5.2.2.4 All tubes and agar plates have observations taken on them within a 24-hour period. In some instances longer incubation periods are required, but observations are taken after each 24 hours of incubation. Only on weekends are results of plate and tube inoculations read after 48 to 72 hours' incubation. A blue-ball point pen is used for marking 24-hour observations; a red pencil for 48-hour observations; a regular lead pencil for 72-hour observations.

5.2.3. Types of confirmatory media and their reactions following inoculation for the Coliforms - Aeromonas series of tests.

- 5.2.3.1. EC broth - two tubes of this medium are inoculated from a P-A bottle; one tube is incubated at  $35^\circ\text{C}$  and another tube is incubated at  $44.5^\circ\text{C}$ , both for a 24-48 hour incubation period. A positive result is scored if gas production is evident in the inverted tube after incubation. If the amount of gas in the inverted tube is less than 10% the result is marked as g; if 10% or more gas is produced, the result is marked as G.

If gas is produced in the  $35^\circ\text{C}$  tube, but no lactose fermenting (pink or red) colonies are found on the MacConkey plate, another MacConkey agar plate is streaked from the  $35^\circ\text{C}$  EC tube. If bacterial growth



occurs in the EC tubes, but no gas production, the result is indicated by +, if no growth, the result is indicated by -. If no growth occurs in either of the EC tubes, but pink or red colonies occur on the MacConkey agar plate, the inoculation of EC tubes from the P-A bottle should be repeated.

- 5.2.3.2. MacConkey agar plates - a loopful of inoculum from each P-A bottle is streaked on one half of each MacConkey agar plate. The plates are inverted after the streaking procedure and placed in a plastic caketette with a tight-fitting lid. The caketette is then placed in the 35 C incubator and the plates are observed after 24 hours for the presence of bacterial colonies.

The bacterial colonies are viewed with the aid of light from the Daylight Fluorescent Titration Illuminator or other suitable device. The colonies are described on the bench sheets as to size and colour. The size may range from 0.5 mm to 3-4 mm depending on the growth characteristics of the organism and whether the MacConkey medium has any inhibitory effect on the cells deposited during the streaking procedure. The colonies may appear translucent and the same colour as the medium or they may be opaque with a creamy appearance exhibiting either no colour or various shades of pink through to dark red. They may also be convex or flat with a mucoid or butyrous consistency.

After descriptions of the different types of colonies are noted on the bench sheet, representative colonies are picked off and each is inoculated into a Lactose Purple broth tube and onto a Nutrient Gelatin agar plate. Preference is given to pink or red, lactose fermenting colonies before picking non-lactose fermenting colonies. Any non-lactose fermenting colonies less than 0.5 mm in size are generally not picked as they are not considered as potential indicators of sanitary pollution.

MacConkey agar plates incubated for periods longer than 24 hours may produce colonial growth which is unsuitable for designating the type of lactose fermentation or for picking colonies to other media. When this occurs, the streaking procedure from the P-A bottle should be repeated.

- 5.2.3.3. Lactose Purple broth - a positive result is scored if gas production is evident in the inverted tube after incubation. Acid production is present when the colour changes from purple to yellow.
- 5.2.3.4. Nutrient Gelatin agar plate - inoculum from the same colony used for inoculation of the Lactose Purple broth tube is used for inoculation of a Nutrient Gelatin agar plate, which is incubated at 35 C for 24-48 hours. After 24 hours, the culture is given an oxidase test.



The test consists of moistening Whatman #3, 9 cm filter paper with a few drops of the tetramethyl-p-phenylenediamine reagent. Using a flamed, platinum loop, a portion of a bacterial colony is removed from the Nutrient Gelatin agar plate and rubbed onto the moistened portion of the filter paper. Within 10-15 seconds, the bacterial area on the filter paper should turn dark purple or black in colour, if the organisms is oxidase positive. The organism is considered as oxidase negative if, after 60 seconds, no colour change or only a pink or mauve colour has formed.

The production of gelatinase will be indicated by a zone of haziness around the bacterial growth in the agar medium within 24-48 hours. A doubtful or negative indication of gelatinase must be checked after 48 hours incubation by flooding the medium surrounding the bacterial growth with a saturated solution of ammonium sulphate  $(\text{NH}_4)_2\text{SO}_4$ . This test should be performed only after the oxidase test has been done.

5.2.4. Types of confirmatory media and their reactions following inoculation for Staphylococcus aureus.

5.2.4.1. Mannitol Salt agar plate - a loopful of inoculum from each P-A bottle is streaked on one half of a Mannitol Salt agar plate. The plates are inverted after the streaking procedure and placed in a plastic cakette with a tight-fitting lid. The cakette is then placed in the 35°C incubator and examined after 24-48 hours for typical Staphylococcus aureus colonies. These colonies are usually 2-3 mm in size, butyrous and bright yellow in colour. The medium surrounding the colony generally changes from a pink to yellow colour. Cultures other than S. aureus may form white or pink colonies. Occasionally bacillus-shaped organisms will form yellow mucoid colonies on the medium. A Gram stain should be performed on each suspected S. aureus culture and the culture should also be tested for catalase and coagulase production.

5.2.4.2. Catalase test - this test is done by first placing a glass microscope slide inside a petri dish. A small amount of inoculum from a colony should be placed in the centre of the glass slide. Using a pipetting device, a drop of 3% hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) is placed on the inoculum. The pipette is withdrawn immediately and the lid of the petri dish is closed down so that the reaction may be observed through the lid of the petri dish. A positive catalase reaction is indicated by an effervescent or bubbling reaction when the  $\text{H}_2\text{O}_2$  comes in contact with the inoculum.

5.2.4.3. Coagulase test - the culture is inoculated into a tube of Trypticase Soy broth, which is incubated at 35°C for 18-24 hours. About 0.05 ml of the 18-24 hour culture is added to a screw-capped (13 x 100 mm) sterile tube, which has had 0.5 ml of reconstituted rabbit plasma added to it. The

tube is generally rotated to ensure a thorough mixing of the contents. The cap is securely tightened on the tube, which is then placed in a 35°C incubator for four hours.

Positive and negative control tubes should be set up at the same time. The tubes will both contain 0.5 ml of rabbit plasma, but one tube will be inoculated with 0.05 ml of a known positive S. aureus culture and the other tube will be inoculated with 0.05 ml of a known negative culture such as E. coli. All tubes are inspected at the end of four hours incubation for clotting of the plasma. If no clotting has occurred, the tubes may be held for another 14 hour period at room temperature. Further incubation may lead to false positive reactions.

Clotting of the known positive S. aureus culture as well as the unknown culture constitutes a positive test for coagulase. Any unused reconstituted plasma may be kept at 4°C for up to 48 hours, if the plasma is kept in a sealed container that prevents evaporation or contamination of the plasma. The known negative culture should preferably be negative for citrate utilization as the plasma may contain citrate.

5.2.5. Types of confirmatory media and their reactions following inoculation for Pseudomonas aeruginosa.

5.2.5.1. Drake's broth - following inoculation from the P-A bottle, this medium is incubated at 41.5°C for up to 72 hours. After each 24 hour period, the Drake's broth is checked for evidence of growth and fluorescence using a long wave (366 nm) ultraviolet light. If no fluorescence occurs in the first 24 hours, the tube is reincubated and checked for fluorescence in the subsequent 24 hour periods. When fluorescence occurs, inoculum is streaked from the Drake's broth onto Skim Milk agar.

5.2.5.2. Skim Milk agar - on this medium, P. aeruginosa grows well forming light green colonies, which fluoresce under the ultraviolet light, and have a definite clearing zone (1-3 mm) in the medium around the colonial growth area.

5.2.5.3. In some instances, P. aeruginosa may be detected directly on MacConkey agar plates, because colonies have a grape-like odour, a blue-green colour, and have a flat, irregular appearance. Inoculum from these types of colonies may be streaked directly on Skim Milk agar plates.

5.2.6. Types of confirmatory media and their reactions following inoculation for fecal streptococci.

5.2.6.1. Ethyl Violet Azide (EVA) broth - this medium is inoculated from the P-A bottle with 2-3 loopfuls of inoculum. The medium is incubated at 35°C for 24-48 hours. Each day the medium is checked for growth. A positive result is confirmed by a dense bacterial suspension. With some cultures, sedimentation of the bacterial suspension may take place giving a blue to purple button-like formation when the tube is viewed from below.

- 5.2.6.2. Enterococcus agar plates - a loopful of inoculum from each P-A bottle is streaked on one half or one quarter of an Enterococcus agar plate. The plates are inverted after the streaking procedure and placed in a plastic caketette with a tight-fitting lid. The caketette is then placed in the 35 C incubator and examined after 24-48 hours for the presence of typical fecal streptococci colonies. These colonies are small, dark red or maroon in colour, usually about 0.5 to 1 mm diameter. A catalase test should be performed on these colonies to ensure the colonies are catalase negative.

A positive result for fecal streptococci is indicated when typical colonies grow on the enterococcus medium and good growth occurs in the EVA broth. If one or the other of the results is negative, but not both, inoculum should be transferred from the medium which gave a positive result to the medium which gave a negative result. If, after the appropriate incubation period, the result is still negative, an overall negative result is given for the presence of fecal streptococci. If the catalase test is positive, the result for fecal streptococci is marked as negative.

- 5.2.7. Types of confirmatory media and their reactions following inoculation for Clostridium perfringens.

- 5.2.7.1. Skim Milk tube - with this test, anaerobic conditions must be established in the tube prior to inoculation. Each Skim Milk tube is placed in a beaker of water filled so that the water level in the beaker is the same height as the medium level in the Skim Milk tube. The beaker of water is brought to a slow boil and kept there for five minutes. The tubes are removed from the boiling water and allowed to cool in a test tube rack until they can be held comfortably by hand (usually about 45 C).

Inoculation of the medium consists of taking a sterile one ml pipette; removing the cap from the P-A bottle; placing the pipette almost to the bottom of the P-A bottle; carefully draw up one ml of inoculum; withdraw the pipette keeping the forefinger securely over the top of the pipette to prevent the inoculum from escaping; replace the cap on the P-A bottle; remove the cap from the Skim Milk tube; place the pipette down to the bottom of the tube; by manipulating the pipette in a rolling fashion with the fingers allow 0.1 ml of inoculum to escape from the pipette; remove the pipette from the Skim Milk tube; replace the cap on the tube; place the pipette and any remaining inoculum into a plastic waste disposal jar containing a Dettol or Wescodyne solution.

The Skim Milk tube is then placed in a test tube rack and incubated at 35 C for 24-48 hours. Each day the tube is examined for clotting of the skim milk and the formation of copious amounts of gas which tears holes in the clotted

milk giving a reaction known as a "stormy fermentation". An inoculating loop is introduced into the tube and a loopful of inoculum is transferred to a clean glass slide; allowed to dry; and the slide is given a Gram stain. If examination of the slide shows the presence of short, plump, rectangular-shaped Gram positive rods with square ends and occurring as single cells or in short chains, these cells are typical for Clostridium perfringens.

The absence of a "stormy fermentation" in the Skim Milk tube and/or the absence of typical C. perfringens cells seen during the microscopic examination is considered as a negative test.

### 5.3. Gram Stain Procedure

- 5.3.1. The preparation of Gram stained slides is required for:
  - 5.3.1.1. Skim Milk tubes having a "stormy fermentation".
  - 5.3.1.2. Suspected Staphylococcus aureus cultures from Mannitol Salt agar plates.
- 5.3.2. Slides prepared from Skim Milk tubes having a "stormy fermentation" should first be marked with an identification number on the frosted glass portion of the slide using a lead pencil. Sometimes, four to six cultures may be put on one slide by using a red wax china-marking pencil to section the slide off into compartments. Two loopfuls of broth inoculum from each Skim Milk tube are placed in each compartment. The second loopful of inoculum is placed on top of the first loopful and spread out slightly. The inoculum should be taken from the "whey" portion of the Skim Milk rather than the coagulated portion. Otherwise the coagulated milk will contribute to difficulties in preparation of properly stained bacteria cells. The inoculum in each compartment is allowed to evaporate to dryness and only then is the slide heat-fixed by passing the inoculated areas on the slide through a bunsen burner flame several times until the glass slide is quite warm, but not too hot to be placed on the back of one's hand.
- 5.3.3. Slides prepared from suspected S. aureus colonies on Mannitol Salt agar plates should first have one or two drops of water placed on the microscope slide. Using a flamed inoculating needle, a small portion of bacterial growth is removed from the same colony as that receiving the catalase test. The inoculating needle is tapped gently into the drop of water on the slide to allow a suspension of bacteria to form in the water. The inoculating needle is flamed and allowed to cool before using it to mix and spread the bacterial suspension into a thin film on the microscope slide. Precautions should be taken to avoid creating too dense a bacterial suspension, which could produce a slide which is difficult to read and interpret. The inoculating needle is flamed again.
- 5.3.4. All slide preparations are air-dried and heat-fixed before beginning the staining procedure. The slides are then taken over to a sink area and placed on a staining rack.

5.3.5. Crystal violet stain is applied to each slide and allowed to cover the area(s) on the slide which contain the bacterial growth. The crystal violet is left in contact with the bacterial cells for one minute. During the above staining period, the cold water tap is turned on to provide the minimum flow of water from the tap that can be obtained without changing to a series of drops. After one minute, each slide is placed in the stream of water about two inches from the orifice of the tap and the crystal violet is quickly washed from the slide.

5.3.6. The slide is placed back on the staining rack and the iodine solution is immediately added to the slide to cover the stained area(s) on the slide. The iodine solution is left in contact with the slide for a minimum period of one minute. If more than one slide is stained at the same time, the iodine solution may be left on the slide indefinitely until each other slide is decolourized and counterstained without any adverse effects.

After one minute, the iodine solution is quickly, but gently washed from the slide and excess water is blotted off the slide with a paper towel. The slide is now ready for the decolourizing step.

5.3.7. After the excess water is blotted from the slide and before the slide has become dry, the 95% alcohol decolourizer is applied to the stained area(s) and the slide is tilted slightly back and forth to allow the alcohol to wash the stained area(s) of the slide. After 30 seconds, the alcohol is rinsed from the slide under the tap water in about 4 to 5 seconds.

5.3.8. The safranin counter stain is quickly added to the slide for a period of about one minute and then it is also quickly washed from the slide. Excess water is blotted from the slide with a paper towel and the slide is allowed to air-dry before making the microscopic examination.

5.3.9. The microscopic examination is done by placing the slide on the microscope stage and positioning the slide so that the light is passing through one of the stained areas on the slide. With one of the low power objectives in place, the microscope is focused until the stained area on the slide is sharply in focus. The objective lens is moved out of position and a drop of immersion oil is placed in the centre of the stained area. The immersion oil lens is moved into position and using the fine adjustment knob, the bacterial cells are carefully brought into focus.

If trouble is experienced in focusing the bacterial cells, the slide should be moved until one of the red wax markings on the slide is brought into view. The red wax marking on the surface of the slide is brought into sharp focus and the slide is carefully moved back towards the stained area of the slide while the viewer attempts to keep the surface of the slide in focus. Care must be taken that sufficient oil is present on the slide to allow this manipulation to take place.

5.3.10. For viewing and interpreting the Gram stain reaction of bacterial cells, the technician should endeavour to locate an area on the slide



in which the bacterial cells are not clumped together but evenly dispersed. Bacterial cells which are deep purple or violet should be considered as Gram positive. Bacterial cells which are red or pink will be Gram negative. Difficulties in determining the Gram reaction of bacterial cells may be resolved somewhat if the light intensity is kept down to a minimum. If this does not work, a repeat Gram stain should be made on a 24-hour plate or broth culture.

#### **5.4. Isolation and Confirmation of Sheen-Colonies from Membrane Filters on m-Endo Agar LES**

- 5.4.1. All drinking water samples, which have had an MF analysis as well as P-A analysis and which have produced sheen colonies on the membrane filter plate, will have one or more of the sheen colonies checked for coliform confirmation.
- 5.4.2. Following enumeration of the sheen and background colonies, the membrane filter plate will be examined for the different sheen colony types, which will be described on the tally sheets as to size and sheen intensity. Colonies selected for isolation should be well isolated from other surrounding colonies.
- 5.4.3. Using a recently flamed, but cooled inoculating needle, the tip of the inoculating needle is gently touched to the top of the sheen colony to allow adherence of some of the colony on the tip of the needle. (The technician should avoid sticking the needle down through the colony until it touches the membrane or attempting to obtain the whole colony on the end of the inoculating needle as other viable but non-multiplying bacteria may be picked up and transferred to the new growth medium.)
- 5.4.4. The inoculating needle containing the inoculum is held in one hand, while the other hand retrieves a previously marked EC tube from the test tube rack. The hand holding the inoculating needle between the thumb and forefinger is used to remove the cap from the EC tube. The top of the EC tube is flamed and the inoculating needle is introduced into the test tube. The tip of the inoculating needle containing the inoculum is immersed below the surface of the EC broth 2-3 times and the needle is withdrawn from the test tube. (Avoid touching the sides of the test tube above the medium during the inoculation procedure.) The cap is replaced on the EC tube, which is then placed back in the test tube rack. A second EC tube is withdrawn from the test tube rack and the inoculation procedure is repeated. The inoculating needle is then carefully flamed to avoid spattering of the inoculum. One of the EC tubes is incubated at 35°C and the other is incubated at 44.5°C for 24-48 hours.
- 5.4.5. After 24 hours incubation, EC tubes showing growth and gas production are streaked on MacConkey agar plates. If gas production is not evident, the tubes are incubated an additional 24 hours. If after the additional 24 hours growth but no gas production is observed in the tubes, only the 35°C tube is streaked on a MacConkey agar plate. MacConkey agar plates are incubated for 24 hours at 35°C.

- 5.4.6. Following incubation, colonies growing on MacConkey agar are described on the tally sheet as to size and colour. Representative colonies are picked off and each is inoculated into a Lactose Purple broth tube and a Nutrient Gelatin agar plate. Preference is given to pink or red, lactose fermenting colonies before picking non-lactose fermenting colonies. Any non-lactose fermenting colonies less than 0.5 mm in size are generally not picked as they are not considered as potential indicators of sanitary pollution.
- 5.4.7. The procedure involved in incubating and taking observations on the inoculations into Lactose Purple broth and Nutrient Gelatin agar will be the same as that described in Section 5.2.3.3. and 5.2.3.4.
- 5.4.8. The interpretation on the results of confirmatory tests on sheen colony isolates will be similar to that for organisms isolated from P-A bottles and will be found in Section 6.

## 6. Interpretation of Presumptive, Confirmatory and the Additional Test Analysis Results

- 6.1. Differentiation and identification of pollution indicator bacteria isolated from P-A bottles depends on evaluation of the results of the confirmatory and additional test analyses. With most of the bacterial groups, one or more analyses must give a positive result before the particular bacterial group is indicated as being present in the original water sample. What constitutes a positive result for each of the respective tests has been described in Sections 5.2.3. to 5.2.7.
- 6.2. The total coliform group is marked as present, if the EC 35°C broth tube and the Lactose Purple broth tube show growth and gas production; lactose fermenting colonies are present on the MacConkey plate; and the colony growth on Nutrient Gelatin agar is oxidase negative.
- 6.3. The fecal coliform group is marked as present, if the EC 44.5°C broth tube and the Lactose Purple broth tube show growth and gas production; lactose fermenting colonies are present on the MacConkey agar plate; and the colony growth on Nutrient Gelatin agar is oxidase negative.
- 6.4. The anaerogenic coliform group is marked as present, if the EC 35°C broth tube and the Lactose Purple broth tube show acid and growth but no gas production; lactose fermenting colonies are present on the MacConkey agar plate; and the colony growth on Nutrient Gelatin agar is oxidase negative.
- 6.5. The Aeromonas group is marked as present, if colony growth on the Nutrient Gelatin agar is gelatinase positive and oxidase positive. The EC 35°C broth tube and Lactose Purple broth tube will usually show growth and no gas production. Colonies on MacConkey agar plates may or may not show lactose fermentation.
- 6.6. The fecal streptococcus or enterococcus group is marked as present, if the EVA broth tube is positive for growth and typical dark red colonies which appear on the Enterococcus agar plate are catalase negative.

- 6.7. Pseudomonas aeruginosa is marked as present, if the Drake's medium exhibits fluorescence; typical blue-green colonies with a grape-like odour are formed on the MacConkey agar plate and the Skim Milk agar has colonies with a green pigment and with hydrolysis of the casein evident around the colonies.
- 6.8. Clostridium perfringens is marked as present, if the Skim Milk tube shows a "stormy fermentation" and typical C. perfringens cells are observed in a Gram stain.
- 6.9. Staphylococcus aureus is marked as present, if butyrous bright yellow colonies form on Mannitol Salt agar plates; a Gram stain shows typical Gram positive cocci; a catalase test is positive and a coagulase tube is positive.
- 6.10. If one or more of the positive characteristics for any pollution indicator group cannot be demonstrated, that group should be considered absent from the sample. P-A bottles, which have doubtful, conflicting or negative results for any of these groups, will have the tally sheet marked with a "presumptive only" result, which is marked by "P".

## 7. Preparation of Media Used in Presence-Absence (P-A) Procedure

### 7.1. MacConkey Broth (with Tryptone) - Presumptive (P-A) Medium

#### 7.1.1. Apparatus Required for Preparation of MacConkey Broth Medium

- 7.1.1.1. Stirring hot plate
- 7.1.1.2. Stainless steel beaker, 7 liter
- 7.1.1.3. Stirring magnet
- 7.1.1.4. Asbestos gloves
- 7.1.1.5. Top loading balance, sensitive to 0.1 g
- 7.1.1.6. Spatula
- 7.1.1.7. Automatic dispenser set at 26 ml
- 7.1.1.8. Racks for 250 ml bottles (5)
- 7.1.1.9. 250 ml bottles plus rubber-lined bakelite caps (120)
- 7.1.1.10. Graduated cylinder, 1 or 2 liter
- 7.1.1.11. Weighing dish

#### 7.1.2. Reagents Required for Preparation of MacConkey Broth Medium

- 7.1.2.1. Dehydrated MacConkey broth (Difco) - 30.0 g/l
- 7.1.2.2. Tryptone (Difco) or Trypticase (BBL) - 5.0 g/l
- 7.1.2.3. Distilled water

#### 7.1.3. Preparation of 6 liters of triple strength medium for 250 ml P-A bottles:

- 7.1.3.1. Measure out 6 liters of distilled water into the stainless steel beaker; place the stirring magnet (alcohol flamed) in the beaker; put the beaker on the stirring hot plate and activate the stirring mechanism to medium speed (without use of heat).



- 7.1.3.2. Check the balance level and zero the balance; place the weighing dish on the balance and tare the balance to zero.
- 7.1.3.3. Using a clean spatula, carefully weigh out 90.0 g of dehydrated Tryptone powder into the weighing dish. Pour the Tryptone powder slowly into the distilled water which is being stirred in the stainless steel beaker.
- 7.1.3.4. Using a clean spatula, carefully weigh out 540.0 g of the dehydrated MacConkey broth medium.
- 7.1.3.5. When the Tryptone has dissolved, slowly add the MacConkey broth powder into the stainless steel beaker, while continuing to stir the Tryptone-water medium.
- 7.1.3.6. The medium is kept stirring continuously until all the MacConkey broth powder has gone into solution (about 10 minutes).
- 7.1.3.7. The dissolved medium is moved to the dispensing area and the automatic dispenser is checked to determine that the dispensed volume of medium per cycle is 26 ml. The medium is now dispensed at the rate of two 26 ml volumes per bottle.
- 7.1.3.8. The bottle caps are placed on the bottles in a loosened position and two racks of bottles are placed in each autoclave for a period of 5 - 10 minutes. This allows for preheating or warming of the bottles and medium before beginning the autoclave cycle.
- 7.1.3.9. The autoclave period is 12 minutes at 121°C. The racks of bottles should be removed from each autoclave as soon as the pressure in the chamber returns to zero. The total time in the autoclave following the start of the cycle should not exceed 30 minutes.
- 7.1.3.10. The racks of bottles should be placed in the refrigerator within 10 minutes after they are removed from the autoclave. When the bottles have cooled after several hours, the caps should be tightened and the bottles may be removed from the refrigerator and stored at room temperature for up to one month.
- 7.1.3.11. The final pH of the medium is  $7.3 \pm 0.2$  at 25°C.

## 7.2. Lactose Lauryl Tryptose Broth - an Alternative Presumptive (P-A Test) Medium

Because supplies of MacConkey broth medium are sometimes difficult to obtain, an alternative formulation for a presumptive medium was devised using Lactose broth and Lauryl Tryptose broth; its preparation is described below.

### 7.2.1. Apparatus Required for Preparation of Lactose Lauryl Tryptose Broth Medium

- 7.2.1.1. Same as in 7.1.1., plus the addition of:
- 7.2.1.2. Analytical balance, sensitive to 0.0001 g

7.2.2. **Reagents Required for Preparation of Lactose Lauryl Tryptose Broth Medium**

- 7.2.2.1. Dehydrated Lactose broth (Difco) - 13.0 g/l
- 7.2.2.2. Dehydrated Lauryl Tryptose broth (Difco) - 17.75 g/l
- 7.2.2.3. Bromocresol Purple (British Drug Houses) (BDH) - 0.0085 g/l
- 7.2.2.4. Distilled water
- 7.2.2.5. Sodium hydroxide (NaOH), reagent grade pellets.
- 7.2.2.6. Sodium Hydroxide Solution (0.1N)  
In a volumetric flask dissolve 4.0 g sodium hydroxide in distilled water and dilute to 1 liter.

7.2.3. **Preparation of 6 liters of triple strength medium for 250 ml P-A bottles:**

- 7.2.3.1. Measure out 6 l of distilled water into the stainless steel beaker; place the stirring magnet (alcohol flamed) in the beaker; put the beaker on the stirring hot plate and activate the stirring mechanism to medium speed (without the use of heat).
- 7.2.3.2. Check the balance level and zero the balance; place the weighing dish on the balance and tare the balance to zero.
- 7.2.3.3. Using a clean spatula, weigh out 319.5 g of Lauryl Tryptose broth. Pour the Lauryl Tryptose powder slowly into the distilled water, which is being stirred continuously in the 7 l beaker.
- 7.2.3.4. Clean the spatula and weigh out 234.0 g of Lactose broth. Slowly add the Lactose broth powder into the distilled water, after the Lauryl Tryptose powder has dissolved.
- 7.2.3.5. Check the analytical balance and zero the balance; place a weighing dish on the balance and tare the balance to zero.
- 7.2.3.6. Weigh out 0.15 g of Bromocresol Purple and add to 10 ml of 0.1N NaOH to dissolve it; add to the Lactose/Lauryl Tryptose broth solution.
- 7.2.3.7. The dissolved medium is dispensed into each P-A bottle at the rate of two 26 ml volumes or one 52 ml volume per bottle. The caps are now loosely placed on the bottles.
- 7.2.3.8. The racks containing the P-A bottles are placed in a preheated autoclave for 5-10 minutes before starting the autoclave period which is 12 minutes at 121°C. Total time in the autoclave should not exceed 30 minutes following the start of the autoclave period.
- 7.2.3.9. The racks of bottles should be placed in a refrigerator within 10 minutes of removing from the autoclave. When the bottles have cooled, the caps should be tightened and the P-A bottles stored at room temperature for 24 hours prior to use.

7.2.3.10. The final pH of the medium is  $6.8 \pm 0.2$  at 25°C.

7.3. **EC Medium** - this medium is used in the confirmatory part of the P-A test scheme for both coliforms and fecal coliforms.

7.3.1. **Apparatus Required for Preparation of EC Medium**

- 7.3.1.1. Stirring hot plate
- 7.3.1.2. Stainless steel beaker, 2 liter
- 7.3.1.3. Stirring magnet
- 7.3.1.4. Asbestos gloves
- 7.3.1.5. Top loading balance, sensitive to 0.1 g
- 7.3.1.6. Spatula
- 7.3.1.7. Automatic dispenser set at 10 ml
- 7.3.1.8. Test tubes, 16 x 150 mm (100 tubes)
- 7.3.1.9. Test tubes, 8 x 45 mm (100 tubes)
- 7.3.1.10. Metal or plastic caps 16 mm (100 caps)
- 7.3.1.11. Test tube baskets
- 7.3.1.12. Graduated cylinder, 1 or 2 liter
- 7.3.1.13. Weighing dish

7.3.2. **Reagents Required for Preparation of EC Medium**

- 7.3.2.1. EC medium (Difco)
- 7.3.2.2. Distilled water

7.3.3. **Preparation of 1 liter of EC medium:**

- 7.3.3.1. Measure out 1 liter of distilled water and pour into the 2 liter beaker.
- 7.3.3.2. Place a stirring magnet (alcohol flamed) in the beaker and place the beaker on the stirring hot plate. Activate the stirrer to medium speed (without use of heat).
- 7.3.3.3. Check balance level and zero the balance. Place the weighing dish on the balance and tare the balance to zero.
- 7.3.3.4. Weigh out 37.0 g of EC medium powder into the weighing dish with a clean spatula.
- 7.3.3.5. Slowly pour the EC powder into the liter of distilled water and continue the stirring action until all the powder is in solution.
- 7.3.3.6. Place in each 16 x 150 mm test tube, a single inverted 8 x 45 mm test tube.
- 7.3.3.7. When the EC medium is in solution, place the beaker next to the pipetting machine and check to see that each cycle of the machine delivers 10 ml.
- 7.3.3.8. Dispense 10 ml of the medium into each test tube and then place caps on each of the tubes.

- 7.3.3.9. Autoclave the baskets containing tubes of EC medium at 121°C for 15 minutes. Remove the baskets from the autoclave as soon as possible after the end of the autoclave cycle.
  - 7.3.3.10. Within 10 minutes, store the medium in the refrigerator for no longer than two weeks before use.
  - 7.3.3.11. Final pH of the medium is  $6.9 \pm 0.2$  at 25°C.
- 7.4. **Drake's Medium** - this medium is used to determine the presence of fluorescent pseudomonads, particularly Pseudomonas aeruginosa. If the medium is used in a replicate tube dilution series, the most probable number (MPN) of fluorescent pseudomonads may be determined.
- 7.4.1. **Apparatus Required for Preparation of Drake's Medium** (similar to 7.3.1., but omitting 7.3.1.8., 7.3.1.9. and 7.3.1.10.)
    - 7.4.1.1. Test tubes, 16 x 150 mm (100 tubes)
    - 7.4.1.2. Metal or plastic caps, 16 mm (100 caps)
  - 7.4.2. **Reagents Required for Preparation of Drake's Medium**
    - 7.4.2.1. Magnesium sulphate ( $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ ), reagent grade
    - 7.4.2.2. Potassium sulphate ( $\text{K}_2\text{SO}_4$ ), reagent grade
    - 7.4.2.3. Di-potassium hydrogen phosphate ( $\text{K}_2\text{HPO}_4$ ), reagent grade
    - 7.4.2.4. l-asparagine
    - 7.4.2.5. Glycerol - Analar
    - 7.4.2.6. Distilled water
  - 7.4.3. Preparation of 1 liter of Drake's medium:
    - 7.4.3.1. Put 1 liter of distilled water into a 2 liter stainless steel beaker. Place a stirring magnet (alcohol flamed) in the beaker and place it on a stirring hot plate. Activate the stirrer to medium speed (without use of heat).
    - 7.4.3.2. Check the balance level and zero the balance. Place a weighing dish on the balance and tare the balance to zero.
    - 7.4.3.3. Weigh out separately and dissolve individually in the liter of distilled water, each of the following ingredients:
      - 0.5 g magnesium sulphate,
      - 10.0 g potassium sulphate,
      - 1.0 g di-potassium hydrogen phosphate,
      - 10.0 g glycerol, (may be more conveniently weighed out into 100 ml beaker than a weighing dish),
      - 2.0 g l-asparagine.
    - 7.4.3.4. When the ingredients have dissolved, dispense 10 ml of the medium into each test tube, cap the tubes and autoclave for 15 minutes at 121°C.

- 7.4.3.5. Remove the baskets of tubes from the autoclave at the end of the autoclave cycle and refrigerate them within 10 minutes for no longer than two weeks.
- 7.4.3.6. Final pH of the medium is  $7.6 \pm 0.2$  at  $25^{\circ}\text{C}$ .
- 7.5. **Ethyl Violet Azide Broth (EVA)** - this medium provides a confirmation test for the presence of fecal streptococci.
  - 7.5.1. **Apparatus Required for Preparation of Ethyl Violet Azide Broth Medium** (similar to 7.3.1. except that 7.3.1.9. is not required)
  - 7.5.2. **Reagents Required for Preparation of Ethyl Violet Azide Broth Medium**
    - 7.5.2.1. Ethyl Violet Azide (EVA) broth (BBL)
    - 7.5.2.2. Distilled water
  - 7.5.3. Preparation of 1 liter of EVA broth:
    - 7.5.3.1. Measure out 1 liter of distilled water into the two liter stainless steel beaker. Place a stirring magnet (alcohol flamed) in the beaker and place it on the stirring hot plate. Activate the stirrer to medium speed (without use of heat).
    - 7.5.3.2. Check the balance level and zero the balance. Place a weighing dish on the balance and tare the balance to zero.
    - 7.5.3.3. Weigh out 35.8 g of the EVA powder into the weighing dish with a clean spatula. Slowly pour the EVA powder into the liter of distilled water and continue the stirring action until all the powder has dissolved.
    - 7.5.3.4. When the ingredients have dissolved, dispense 10 ml of the medium into each test tube using the automatic pipetting machine.
    - 7.5.3.5. Cap the tubes and autoclave the medium for 15 minutes at  $121^{\circ}\text{C}$ . Remove the baskets of tubes from the autoclave as soon as the cycle is finished and refrigerate them within 10 minutes for no longer than two weeks before using.
    - 7.5.3.6. Final pH of the medium is  $7.0 \pm 0.2$  at  $25^{\circ}\text{C}$ .
- 7.6. **Skim Milk Broth** - this medium determines the presence of Clostridium perfringens, which give a "stormy fermentation" reaction in the clotted milk. Anaerobic conditions must be established in the medium prior to use.
  - 7.6.1. **Apparatus Required for Preparation of Skim Milk Broth Medium**
    - 7.6.1.1. Stirring hot plate
    - 7.6.1.2. Stainless steel beaker, 2 liter
    - 7.6.1.3. Stirring magnet
    - 7.6.1.4. Asbestos gloves
    - 7.6.1.5. Top loading balance, sensitive to 0.1 g

- 7.6.1.6. Spatula
- 7.6.1.7. Automatic dispenser set at 10 ml
- 7.6.1.8. Test tubes, 18 x 150 mm (50 tubes)
- 7.6.1.9. Metal or plastic caps, 18 mm (50 caps)
- 7.6.1.10. Iron nails, 1" long (50 nails)
- 7.6.1.11. Test tube baskets
- 7.6.1.12. Graduated cylinder, 1 or 2 liter
- 7.6.1.13. Weighing dish

**7.6.2. Reagents Required for Preparation of Skim Milk Broth Medium**

- 7.6.2.1. Skim Milk (Difco)
- 7.6.2.2. Distilled water
- 7.6.2.3. Xylene

**7.6.3. Preparation of 1 liter of Skim Milk broth:**

- 7.6.3.1. The iron nails should be soaked in xylene for 24 hours; rinsed several times in distilled water to remove all of the xylene; and placed in an oven overnight to dry them off. One nail should be placed in each test tube.
- 7.6.3.2. Put 1 liter of distilled water into the stainless steel beaker. Place a stirring magnet in the beaker and place it on a stirring hot plate. Activate the stirring mechanism to medium speed (without use of heat).
- 7.6.3.3. Check the balance level and zero the balance. Place a weighing dish on the balance and tare the balance to zero.
- 7.6.3.4. Weigh out 100 g of the Skim Milk powder into the weighing dish using a clean spatula.
- 7.6.3.5. Using the spatula, gradually add the Skim Milk powder to the distilled water and continue the stirring action until all of the powder has dissolved.
- 7.6.3.6. Using the automatic dispenser, two 10 ml volumes of the Skim Milk broth are dispensed into each test tube to give a final volume of 20 ml.
- 7.6.3.7. The tubes are capped and the medium is autoclaved at 121° for 12 minutes. The baskets of tubes are removed quickly at the end of the autoclave cycle and placed in a refrigerator within ten minutes. The tubes should be used within two weeks and the medium has to be boiled before use to achieve anaerobic conditions suitable for C. perfringens.
- 7.6.3.8. The final pH is 6.4 ± 0.2 at 25°C.

**7.7. Lactose Purple Broth** - this medium is used for determining lactose fermentation following inoculation with a pure culture of an organism.

**7.7.1. Apparatus Required for Preparation of Lactose Purple Broth Medium**

- 7.7.1.1. Stirring hot plate
- 7.7.1.2. Stainless steel beaker, 2 liter
- 7.7.1.3. Stirring magnet
- 7.7.1.4. Asbestos gloves
- 7.7.1.5. Top loading balance, sensitive to 0.1 g
- 7.7.1.6. Spatula
- 7.7.1.7. Automatic dispenser set at 10 ml
- 7.7.1.8. Test tubes, 16 x 150 mm (100 tubes)
- 7.7.1.9. Metal or plastic caps, 16 mm (100 caps)
- 7.7.1.10. Test tube baskets
- 7.7.1.11. Graduated cylinder
- 7.7.1.12. Test tubes, 8 x 45 mm (100 tubes)
- 7.7.1.13. Weighing boats

**7.7.2. Reagents Required for Preparation for Lactose Purple Broth Medium**

- 7.7.2.1. Tryptone (Difco) or Trypticase (BBL)
- 7.7.2.2. Purple Broth base (Difco)
- 7.7.2.3. Lactose (Difco)
- 7.7.2.4. Distilled water

**7.7.3. Preparation of 1 liter of Lactose Purple broth:**

- 7.7.3.1. Put 1000 ml of distilled water into the stainless steel beaker. Place a stirring magnet in the beaker and place it on the stirring hot plate. Activate the stirring mechanism to run at medium speed (without use of heat).
- 7.7.3.2. Check the balance level and zero the balance. Place the weighing dish on the balance and tare the balance to zero.
- 7.7.3.3. Weigh out 5.0 g of tryptone powder with a clean spatula. Slowly pour the tryptone powder into the 1000 ml of distilled water. Weigh out 10.0 g of lactose and add slowly to the contents of the beaker. Weigh out 16.0 g of the Purple Broth Base and add slowly to the contents of the beaker. Continue the stirring of the medium until the ingredients are in solution and then dispense into the test tubes in 10 ml volumes.
- 7.7.3.4. Before dispensing the medium, each 16 x 150 mm test tube has a single inverted 8 x 45 mm test tube inserted into it.
- 7.7.3.5. Cap the tubes and place the baskets of media in the autoclave. Sterilize the medium for 15 minutes at 121 °C. Remove the baskets at the end of the autoclave cycle and refrigerate within 10 minutes. The medium should be used within a two-week period.

7.7.3.6. The final pH of the medium is  $7.0 \pm 0.2$ .

7.8. **Trypticase Soy Broth** - this medium is used for culturing organisms, when an inoculum is required for streaking plates or inoculation of other media or reagents, such as the coagulase test.

7.8.1. **Apparatus Required for Preparation of Trypticase Soy Broth Medium**

- 7.8.1.1. Stirring hot plate
- 7.8.1.2. Stainless steel beaker, 2 liter
- 7.8.1.3. Stirring magnet
- 7.8.1.4. Asbestos gloves
- 7.8.1.5. Top loading balance, sensitive to 0.1 g
- 7.8.1.6. Spatula
- 7.8.1.7. Automatic dispenser device set at 10.0 ml
- 7.8.1.8. Test tubes, 16 x 150 mm (100 tubes)
- 7.8.1.9. Metal or plastic caps, 16 mm (100 caps)
- 7.8.1.10. 3 test tube baskets
- 7.8.1.11. Graduated cylinder, 1 or 2 liter
- 7.8.1.12. Weighing dish

7.8.2. **Reagents Required for Preparation of Trypticase Soy Broth Medium**

- 7.8.2.1. Trypticase Soy broth (BBL)
- 7.8.2.2. Distilled water

7.8.3. **Preparation of 1 liter of Trypticase Soy broth**

- 7.8.3.1. Measure out 1 liter distilled water and pour into the 2 liter stainless steel beaker.
- 7.8.3.2. Place a stirring magnet (alcohol flamed) in the beaker and put the beaker on the stirring hot plate. Activate the stirring mechanism to medium speed.
- 7.8.3.3. Check balance level and zero the balance. Place the weighing dish on the balance and tare the balance to zero.
- 7.8.3.4. Weigh out 30.0 g of the Trypticase Soy broth powder using a clean spatula into the weighing dish.
- 7.8.3.5. Slowly pour the powder medium into the beaker of distilled water and continue the stirring action (without heating) until the powder is in solution.
- 7.8.3.6. Using the automatic dispensing device, add 10.0 ml of the medium to each test tube. Cap the test tubes and place the baskets of test tubes in the autoclave.
- 7.8.3.7. Autoclave the Trypticase Soy broth medium at  $121^{\circ}\text{C}$  for 15 minutes. At the end of the autoclave cycle, remove the baskets of test tubes and place in a refrigerator within 10 minutes. Final pH of the medium is  $7.3 \pm 0.2$ .



**7.9. MacConkey Agar Plates** - this medium is used for isolation of Gram negative bacteria and is particularly useful for determining lactose reactions of coliform bacteria.

**7.9.1. Apparatus Required for Preparation of MacConkey Agar Plate Medium**

- 7.9.1.1. Stirring hot plates
- 7.9.1.2. Stainless steel beaker, 2 liter
- 7.9.1.3. Stirring magnet
- 7.9.1.4. Asbestos gloves
- 7.9.1.5. Top loading balance, sensitive to 0.1 g
- 7.9.1.6. Spatula
- 7.9.1.7. Graduated cylinder, 1 liter
- 7.9.1.8. Weighing dish
- 7.9.1.9. Thermometer (0-110°C)
- 7.9.1.10. Petri plates, 100 x 15 mm square, sterile, plastic
- 7.9.1.11. Plastic cakettes with lids
- 7.9.1.12. Beaker, 500 ml, sterile
- 7.9.1.13. Aluminum foil
- 7.9.1.14. Electric fan
- 7.9.1.15. Wescodyne 5%
- 7.9.1.16. Laminar Flow Unit

**7.9.2. Reagents Required for MacConkey Agar Plate Medium**

- 7.9.2.1. MacConkey agar (Difco)
- 7.9.2.2. Distilled water

**7.9.3. Preparation of 1 liter of MacConkey agar medium:**

- 7.9.3.1. Measure out 1 liter of distilled water into the stainless steel beaker. Place a stirring magnet (alcohol flamed) in the beaker and put it on a stirring hot plate, which has the stirring mechanism set for medium speed.
- 7.9.3.2. Check the balance level and zero the balance. Place a weighing dish on the balance and tare the balance to zero.
- 7.9.3.3. Using the spatula, weigh out 50.0 g of the MacConkey agar powder and slowly add it to the distilled water being stirred in the beaker. Cover the mouth of the beaker with aluminum foil and insert a thermometer through the foil and attach the thermometer to the side of the beaker. Heat the medium to a slow boil or until the agar medium has gone into solution (90-92°C).
- 7.9.3.4. Remove the thermometer and place the beaker of dissolved medium in the autoclave for a period of 15 minutes at 121°C. At the end of the autoclave cycle, using

asbestos gloves, place the beaker of sterilized medium on a "cold" stirring hot plate.

- 7.9.3.5. Set the stirring mechanism to medium speed and position the electric fan beside the beaker. Turn on the fan to assist with cooling the medium to about 50-55°C. A thermometer which has been kept in 95% ethyl alcohol is removed from the alcohol and allowed to drip dry. The thermometer with a clip attachment is fastened in an aseptic manner inside the beaker (through the aluminum foil) until the temperature has cooled sufficiently for pouring plates.
- 7.9.3.6. During the cooling period, the bench of the laminar flow hood is swabbed with Wescodyne disinfectant and the petri dishes are laid out in four or five rows.
- 7.9.3.7. When the MacConkey medium has reached 50-55°C, the thermometer is removed and the sterile 500 ml beaker is three-quarters filled with the medium. The 500 ml beaker provides a more convenient device for pouring plates than the large liter beaker.
- 7.9.3.8. Using aseptic procedure, about 25 ml of the medium is poured into each petri dish. The lids are left slightly open (about 10%) to facilitate solidification of the medium and avoid excess condensation of moisture on the lids of the petri plates. After the medium has solidified, the lids are closed and the petri dishes are inverted and left overnight on a bench to allow drying of the surface of the medium.
- 7.9.3.9. The following day, the petri dishes, still in an inverted position, are placed in plastic cakettes and stored in the refrigerator for use within four weeks. The final pH of the medium is  $7.1 \pm 0.2$ .

**7.10. Enterococcus Agar Plates** - this medium provides a confirmatory test for the presence of fecal streptococci isolated from the presumptive positive P-A bottles.

**7.10.1. Apparatus Required for Preparation of Enterococcus Agar Plate Medium**

- 7.10.1.1. Stirring hot plates
- 7.10.1.2. Erlenmeyer flask, 2 liter, sterile
- 7.10.1.3. Graduated cylinder, 1 liter, sterile
- 7.10.1.4. Beaker, 500 ml, sterile
- 7.10.1.5. Stirring magnet
- 7.10.1.6. Asbestos gloves
- 7.10.1.7. Top loading balance, sensitive to 0.1 g
- 7.10.1.8. Spatula
- 7.10.1.9. Thermometer (0-110°C)
- 7.10.1.10. Large forceps

- 7.10.1.11. Petri dishes, 100 x 15 mm, square, sterile, plastic (40 plates)
- 7.10.1.12. Plastic cakettes with lids
- 7.10.1.13. Aluminum foil
- 7.10.1.14. Electric fan
- 7.10.1.15. 5% Wescodyne
- 7.10.2. **Reagents Required for Preparation of Enterococcus Agar Plate Medium**
  - 7.10.2.1. m-Enterococcus agar (Difco)
  - 7.10.2.2. 1 liter sterile distilled water in Erlenmeyer flask
  - 7.10.2.3. 95% ethyl alcohol
- 7.10.3. **Preparation of 1 liter of m-Enterococcus agar medium:**
  - 7.10.3.1. Check the balance level and zero the balance. Place the sterile 2 liter Erlenmeyer flask on the balance and tare the balance to zero.
  - 7.10.3.2. Using a spatula which has been previously dipped in alcohol and flamed, weigh out 42.0 g of the m-Enterococcus agar powder directly into the sterile flask. Similarly, dip a stirring magnet into 95% alcohol, flame it and transfer it to the sterile flask using a pair of large forceps. (Make sure the flame is out, before placing the magnetic stirrer in the flask.)
  - 7.10.3.3. Place the flask on a stirring hot plate and add about half of the liter of sterile distilled water. Activate the stirring mechanism to medium speed to promote the mixing of the water and powder and then slowly add the remainder of the liter of water.
  - 7.10.3.4. Check to see that none of the medium is stuck to the bottom of the flask and then turn on the heating element to raise the temperature to 92°C, while continuing the stirring action of the medium. An aluminum foil cover should be present over the top of the flask during the entire procedure and a thermometer previously swabbed with 95% alcohol should be attached to the side of the flask during the heating period.
  - 7.10.3.5. As the temperature reaches 92°C, bubbles will form indicating the medium is on the verge of boiling. The heat should be turned off at this point, but the flask should be left on the hot plate while continuing the stirring action until it is ascertained that the medium is a clear, straw colour indicating the agar has gone into solution.
  - 7.10.3.6. The flask is then transferred to a "cold" stirring hot plate, which has the stirring mechanism activated to medium speed. The electric fan is moved into position and turned on to assist in cooling the medium quickly.

- 7.10.3.7. During the cooling period, the bench of the laminar flow hood is swabbed with the 5% Wescodyne disinfectant and the petri dishes are laid out in four or five rows.
- 7.10.3.8. When the medium has reached 50-55°C, the thermometer is removed and the sterile 500 ml beaker is three-quarters filled with the medium for the purpose of obtaining a convenient device for pouring plates.
- 7.10.3.9. Using aseptic procedure, about 25 ml of the medium is poured into each petri dish. The lids are left slightly open (about 10%) to facilitate solidification of the medium and avoid excess moisture condensation on the petri dish lids. After the medium has solidified, the lids are closed and the petri dishes are inverted and left overnight on a bench to allow drying of the surface of the medium.
- 7.10.3.10. The following day, the petri dishes are placed in the plastic cakettes in an inverted position and stored in the refrigerator for use within two weeks. The final pH of the medium is  $7.2 \pm 0.2$ .

**7.11. Nutrient Gelatin Agar Plates** - this medium is used for determining which organisms produce significant gelatinase within a 48 hour period. Also inoculum from this medium is used to test for oxidase production.

**7.11.1. Apparatus Required for Preparation of Nutrient Gelatin Agar Plate Medium**

- 7.11.1.1. Stirring hot plates
- 7.11.1.2. Stainless steel beakers, 1 liter
- 7.11.1.3. Stirring magnet
- 7.11.1.4. Asbestos gloves
- 7.11.1.5. Top loading balance, sensitive to 0.1 g
- 7.11.1.6. Spatula
- 7.11.1.7. Graduated cylinder, 1 liter
- 7.11.1.8. Weighing dish
- 7.11.1.9. Thermometer (0-110 °C)
- 7.11.1.10. Petri plates, 100 x 15 mm round, glass or plastic, (50 plates)
- 7.11.1.11. Plastic cakettes with lids
- 7.11.1.12. Aluminum foil
- 7.11.1.13. Electric fan
- 7.11.1.14. Beaker, glass, 500 ml, sterile
- 7.11.1.15. Wescodyne 5%

**7.11.2. Reagents Required for Preparation of Nutrient Gelatin Agar Plate Medium**

- 7.11.2.1. Nutrient agar (Difco)

- 7.11.2.2. Gelatin (Difco)
- 7.11.2.3. Yeast Extract (Oxoid)
- 7.11.2.4. Distilled water
- 7.11.3. Preparation of 1 liter of Nutrient Gelatin agar medium:
  - 7.11.3.1. Measure out 500 ml of distilled water into a stainless steel beaker. Place a stirring magnet (alcohol flamed) in the beaker and put it on a stirring hot plate which has the stirring mechanism set for medium speed.
  - 7.11.3.2. Check the balance level and zero the balance. Place a weighing dish on the balance and tare the balance to zero.
  - 7.11.3.3. Using the spatula, weigh out 23 g of Nutrient agar powder into the weighing dish and slowly add to the contents of the beaker. Turn on the heating element; fasten a thermometer to the inside of the beaker; and bring the temperature up to about 30-40 °C.
  - 7.11.3.4. Weigh out 3.0 g of Yeast Extract and add it to the Nutrient agar medium.
  - 7.11.3.5. Weigh out 30.0 of gelatin powder into a second stainless steel beaker containing 500 ml of distilled water. Place on a stirring hot plate, activate the stirring mechanism and the heating element until the temperature reaches 30-40 °C and the gelatin has dissolved. Add the gelatin solution to the beaker containing the Nutrient Agar and Yeast Extract.
  - 7.11.3.6. Cover the mouth of the beaker with aluminum foil and with the thermometer still in place continue heating the medium until the temperature has reached 90-92 °C. When the medium has a clear appearance, indicating that all the ingredients have dissolved, the thermometer is removed, fresh aluminum foil is placed over the mouth of the beaker, which is then transferred to the autoclave.
  - 7.11.3.7. The autoclaving time is 15 minutes at 121 °C. The beaker is removed from the autoclave at the end of the cycle using asbestos gloves and placed on a "cold" stirring hot plate. An alcohol-disinfected thermometer is again inserted into the beaker; the stirring mechanism is set at medium speed; the electric fan is turned on beside the beaker and the temperature is allowed to drop to 50-55 °C.
  - 7.11.3.8. During the cooling period, the bench of the laminar flow unit is swabbed with 5% Wescodyne and the petri plates are laid out in four to five rows.
  - 7.11.3.9. When the temperature has cooled sufficiently for pouring, the thermometer is removed and the sterile 500 ml beaker is three-quarters filled with the sterile medium. This beaker is then used for pouring about 20 ml of medium into each petri dish. The lids are left slightly open (about 10%) to facilitate cooling of the medium and avoid excess condensation on the lids of the petri dishes. When the

medium has cooled and solidified, the lids are closed and the petri dishes are inverted and left overnight on the bench to allow drying of the surface of the medium.

- 7.11.3.10 The following day, the petri dishes are placed in the cakettes in an inverted position and stored in the refrigerator for use within two weeks. The final pH of the medium is 6.8.  $\pm 0.2$ .

**7.12. Skim Milk Agar Plates** - this medium is used for determining a number of the characteristics associated with Pseudomonas aeruginosa such as pigmentation, caseinase production, fluorescence, and oxidase reaction.

**7.12.1. Apparatus Required for Preparation of Skim Milk Agar Plates**

- 7.12.1.1. Stirring hot plates
- 7.12.1.2. Stainless steel beaker, 2 liter
- 7.12.1.3. Stainless steel beaker, 1 liter
- 7.12.1.4. Stirring magnets
- 7.12.1.5. Asbestos gloves
- 7.12.1.6. Top loading balance, sensitive to 0.1 g
- 7.12.1.7. Spatula
- 7.12.1.8. Graduated cylinder, 1 liter
- 7.12.1.9. Weighing dish
- 7.12.1.10. Thermometer (0-110 °C)
- 7.12.1.11. Petri dishes, 100 x 15 mm round or square plastic (40 dishes)
- 7.12.1.12. Plastic cakettes with lids
- 7.12.1.13. Aluminum foil
- 7.12.1.14. Electric fan
- 7.12.1.15. Beaker, glass, 500 ml, sterile
- 7.12.1.16. Wescodyne 5%

**7.12.2. Reagents Required for Preparation of Skim Milk Agar Plates**

- 7.12.2.1. Dehydrated Skim Milk powder
- 7.12.2.2. Agar (Difco)
- 7.12.2.3. Distilled water

**7.12.3. Preparation of 1 liter of Skim Milk agar:**

- 7.12.3.1. Measure out 500 ml of distilled water into the 2 liter beaker. Place a stirring magnet (alcohol flamed) in the beaker and put it on a stirring plate which has the stirring mechanism set for medium speed.
- 7.12.3.2. Check the balance level and zero the balance. Place a weighing dish on the balance and tare the balance to zero.

- 7.12.3.3. Weigh out 100 g of Skim Milk powder and add it slowly to the 500 ml of distilled water. Allow the mixture to stir without heat for approximately 30 minutes.
- 7.12.3.4. During this period, measure out 500 ml of distilled water into the 1 liter beaker. Place a stirring magnet (alcohol flamed) in the beaker and put it on a stirring hot plate, which has the stirring mechanism set at medium speed.
- 7.12.3.5. Using a spatula, weigh out 15.0 g of agar and slowly add the agar to the 500 ml of distilled water. Turn on the heating element, cover the beaker mouth with aluminum foil, place a thermometer on the inside of the beaker, and allow the temperature of the medium to slowly rise to 90-92°C. This usually takes 10-12 minutes.
- 7.12.3.6. Both the beaker containing the dissolved agar and the beaker containing the dissolved Skim Milk are covered with aluminum foil and placed in the autoclave for sterilization at 121°C for 12 minutes. Both beakers are removed from the autoclave immediately and placed on stirring plates until the temperature of each has cooled to approximately 55°C.
- 7.12.3.7. At the start of the cooling period, the laminar flow hood is swabbed with 5% Wescodyne and the petri dishes are laid out in four to five rows.
- 7.12.3.8. The Skim Milk solution is then aseptically added to the agar solution and the mixture is stirred for an additional two to three minutes until the temperature has dropped to 50-52°C.
- 7.12.3.9. When the temperature has cooled sufficiently for pouring plates, the sterile 500 ml beaker is three-quarters filled with the Skim Milk medium. This beaker is used for pouring about 20-25 ml of medium into each petri dish. The lids are left slightly open to permit cooling of the medium without excess condensation on the lids of the petri dishes. When the medium has solidified, the lids are closed and the petri dishes are inverted. They are then placed in plastic cakettes into the refrigerator for use within two weeks. The final pH of the medium is  $6.4 \pm 0.2$ .

**7.13. Mannitol Salt Agar Plates** - this medium is used for the isolation and differentiation of Staphylococcus aureus.

**7.13.1. Apparatus Required for Preparation of Mannitol Salt Agar Plates**

- 7.13.1.1. Stirring hot plates
- 7.13.1.2. Stainless steel beaker 2 liter
- 7.13.1.3. Stirring magnet
- 7.13.1.4. Asbestos gloves
- 7.13.1.5. Top loading balance, sensitive to 0.1 g
- 7.13.1.6. Spatula



- 7.13.1.7. Graduated cylinder 1 liter
- 7.13.1.8. Weighing dish
- 7.13.1.9. Thermometer (0-110 °C)
- 7.13.1.10. Petri dishes, 100 x 15 mm, round or square plastic (40 dishes)
- 7.13.1.11. Plastic cakettes with lids
- 7.13.1.12. Aluminum foil
- 7.13.1.13. Electric fan
- 7.13.1.14. Beaker, 500 ml, sterile
- 7.13.1.15. Wescodyne 5%

## 7.13.2. Reagents Required for Preparation of Mannitol Salt Agar Plates

- 7.13.2.1. Dehydrated Mannitol Salt agar (Difco)
- 7.13.2.2. Distilled water

## 7.13.3. Preparation of 1 liter of Mannitol Salt agar:

- 7.13.3.1. Measure out 1 liter of distilled water into the 2 liter beaker. Place a stirring magnet (alcohol flamed) in the beaker and put it on a stirring hot plate, which has the stirring mechanism activated to medium speed.
- 7.13.3.2. Check the balance level and zero the balance. Place a weighing dish on the balance and tare the balance to zero.
- 7.13.3.3. Using a clean spatula, weigh out 111.0 g of the Mannitol Salt agar powder into the weighing dish. Slowly add the powder to the distilled water stirring in the 2 liter beaker. Turn on the heating element and when the powder has been thoroughly wetted, cover the beaker with aluminum foil, place a thermometer in the beaker and allow the contents of the beaker to heat up to 90-92°C. At this time, the thermometer is removed from the beaker, the hole in the aluminum foil is patched with a small piece of autoclave tape and the beaker is placed in the autoclave for sterilization at 121°C for 15 minutes.
- 7.13.3.4. At the end of the autoclave cycle, the beaker is removed, using asbestos gloves, and placed on a "cold" stirring hot plate. The stirring mechanism is set at medium speed and a fan is moved into position to assist with cooling the temperature down to 50-55°C. An alcohol-disinfected thermometer is used to periodically check the temperature in the beaker.
- 7.13.3.5. During the cooling period, the laminar flow unit is switched on for a 30 minute period; the bench of the laminar flow hood is swabbed with 5% Wescodyne and the petri dishes are laid out.
- 7.13.3.6. When the temperature has cooled sufficiently for pouring plates, the sterile 500 ml beaker is three-quarters filled with the Mannitol Salt Agar. This beaker is used for



pouring 20-25 ml of medium into each petri dish. The lids are left slightly open to permit cooling of the medium without excess condensation occurring on the lids of the petri dishes. When the medium has solidified, the lids are closed and the petri dishes are inverted. They are placed in plastic cakettes into the refrigerator for use within two weeks. The final pH of the medium is  $7.4 \pm 0.2$ .

7.14. **Oxidase Reagent** is used to determine if the organism under examination is oxidase positive.

7.14.1. **Apparatus Required for Preparation of Oxidase Reagent**

- 7.14.1.1. Graduated cylinder, 100 ml
- 7.14.1.2. Beaker, 250 ml
- 7.14.1.3. Dark glass stoppered bottle (150 ml), sterile
- 7.14.1.4. Hot plate
- 7.14.1.5. Spatula
- 7.14.1.6. Top loading balance, sensitive to 0.1 g

7.14.2. **Reagents Required for Preparation of Oxidase Reagent**

- 7.14.2.1. N,N,N',N'-tetramethyl-p-phenylenediamine dihydrochloride - (J. T. Baker)
- 7.14.2.2. L-ascorbic acid - (BDH)
- 7.14.2.3. Sterile distilled water

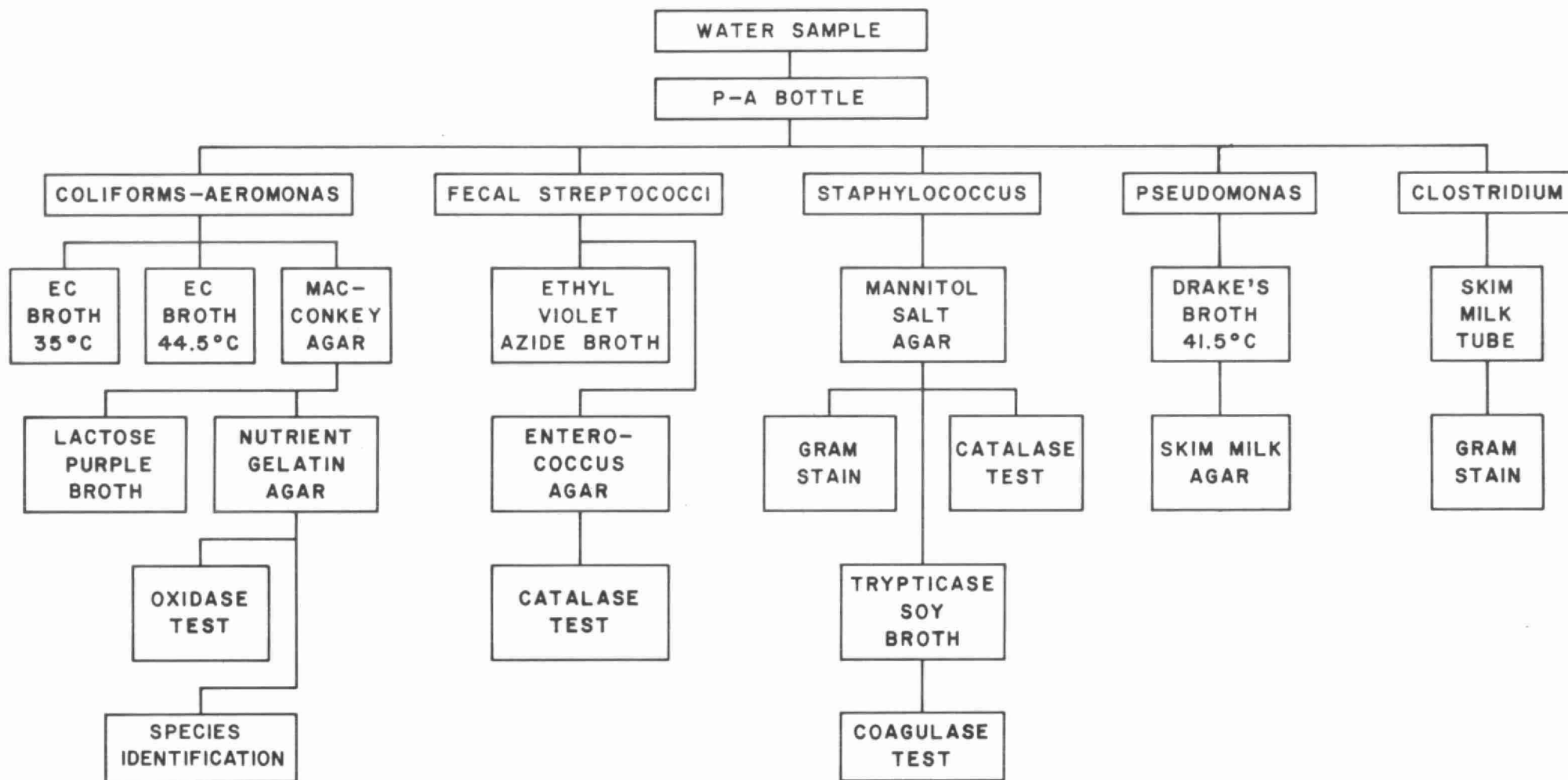
7.14.3. **Preparation of 100 ml of oxidase reagent:**

- 7.14.3.1. Measure out 100 ml of distilled water into sterile beaker.
- 7.14.3.2. Add 0.1 g of ascorbic acid to the distilled water.
- 7.14.3.3. Add 1.0 g of N,N,N',N'-tetramethyl-p-phenylenediamine dihydrochloride to the ascorbic acid solution, and dissolve it with gentle warming if necessary (no higher than 35 °C). Store in dark glass-stoppered bottle at 4 °C.
- 7.14.3.4. Before using, allow the solution to stand at room temperature for 15 minutes.

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PRESENCE - ABSENCE (P-A) TEST SCHEME

## THE ENUMERATION, ISOLATION, AND CONFIRMATION OF PSEUDOMONAS AERUGINOSA

Pseudomonas aeruginosa is an opportunistic pathogen, and has been linked as the causative agent of numerous infections that may be transmitted through a contaminated water supply to a susceptible host. Of chief concern to bathers is the association between P. aeruginosa and otitis externa which has been established in recent years (8.1, 8.2). The presence of P. aeruginosa in swimming waters may contribute significantly to the incidence of outer ear infections in swimmers. In addition to its direct pathogenicity, the association of P. aeruginosa with human fecal waste indicates that where elevated levels of P. aeruginosa are found, serious health hazards may exist due to the presence of other pathogens.

### Sample Handling and Preservation

Bacteriological samples should be collected in Ministry of the Environment (MOE) sterilized glass bottles. The sample bottles should be chilled on ice, and transported to the laboratory, where analysis should commence within 24 hours of the sampling time. Samples containing chlorine as a disinfecting agent should be collected in sterile sampling bottles to which sodium thiosulphate has been added to neutralize any chlorine present.

Depth samples should be taken using sterile sampling bulbs. The same transportation factors apply to the depth samples as to the surface samples.

### Selection of Method

The two procedures commonly used to detect and quantify pollution indicator bacteria are the Most Probable Number (MPN) method and the Membrane Filter (MF) method. The MF method gives direct counts, better reproducibility of results, and allows the examination of larger volumes of sample than the MPN method. However, the examination of water samples with high turbidity due to algae or other material may require testing of these samples by the MPN method. The MF method is less time-consuming than the MPN methodology and the preparation and analysis cost per MF analysis is also less than for MPN analysis.

The mPA medium of Levin and Cabelli (8.3) incorporates four antibiotics and is incubated at  $41.5 \pm 0.5^{\circ}\text{C}$  for 48 hours. These factors inhibit the growth of most heterotrophic bacteria, with the exception of P. aeruginosa.

## PSEUDOMONAS AERUGINOSA AND BACKGROUND COUNTS

### MEMBRANE FILTER ANALYSIS

#### SUMMARY

Matrix.	This method is used on drinking water, surface water and sewage samples.
Organisms determined	The test is designed to measure the number of <u>Pseudomonas aeruginosa</u> bacteria and background bacteria present in water samples from a variety of sources.
Interpretation of results	The presence of <u>Pseudomonas aeruginosa</u> in surface waters is a potential health hazard and indicates the presence of other pathogenic organisms. Its presence in high concentrations (10/100 ml) is indicative of recent fecal pollution.
Principle of method	Each bacterial cell deposited on the membrane filter and given a suitable nutrient source has the potential to grow and multiply sufficiently to form a bacterial colony with distinguishing characteristics.
Time required for analysis	The analysis time for one sample is approximately 5 minutes. The incubation time for <u>P. aeruginosa</u> is $48 \pm 2$ hours. When confirmation tests are desired, they may take an additional 48-72 hours.
Range of application	The analysis can usually be done for sample volumes of 100 ml or less.
Standard deviation	Performance characteristics are not yet available.
Accuracy	Performance characteristics are not yet available.
Detection criteria	Performance characteristics are not yet available.
Interferences and shortcomings	Improper choice of dilutions for membrane filters will affect the ability to obtain plates suitable for counting. High populations of background organisms and toxic substances in the sample or membrane filters will interfere with the development of typical <u>P. aeruginosa</u> colonies.
Minimum volume of sample	A minimum of 100 ml of sample is preferred.

**Preservation and  
sample container**

MOE 250 ml sterile glass bottles should be used for collection of samples. Samples should be iced or refrigerated from the time of sampling to the time of arrival at the laboratory and should arrive within 24 hours of the sampling time.

**Safety  
considerations**

Bacteriological samples, particularly those from sources of water suspected of pollution, may contain pathogenic organisms, and should be handled in a manner to prevent contamination of the sampler and the analyst. P. aeruginosa is an opportunistic pathogen and all workers involved in the enumeration, isolation or confirmation of this organism must be aware of the inherent danger it represents.

## PSEUDOMONAS AERUGINOSA

### Membrane Filter Analysis

#### 1. Introduction

The determination of P. aeruginosa by membrane filtration employs the mPA medium of Levin and Cabelli (8.3). The antibiotics employed in the medium, and the elevated incubation temperature, inhibit the growth of most heterotrophic bacteria other than P. aeruginosa. Typical P. aeruginosa colonies should be confirmed utilizing the techniques detailed.

#### 2. Interferences and Shortcomings

For certain samples, bacterial cells may have been exposed to adverse environmental factors which lower their probability for survival and growth on a membrane filter medium. This effect may be pronounced in this methodology due to the presence of antibiotics and the elevated incubation temperature.

The selection of an appropriate dilution volume is essential. Too small a dilution volume may fail to detect any P. aeruginosa organisms, while too large a volume may cause an overabundance of colonies which would interfere with an accurate count.

The preparation of the mPA medium is quite involved, requiring the precise weighing of small quantities of antibiotics. The pigmentation and colouration of colonies on mPA is variable, resulting in an inherent error in the enumeration.

P. aeruginosa is pathogenic. All workers involved in the enumeration, isolation, or confirmation of this organism must be aware of its potential danger.

#### 3. Apparatus

##### 3.1 Sterile Equipment

- 3.1.1. Pipettes: 1 ml for 1 ml deliveries  
10 ml for 5 and 10 ml deliveries.
- 3.1.2. Graduated cylinders: 100 ml for 100 ml volumes  
50 ml for 50 and 25 ml volumes.
- 3.1.3. 47 mm membrane filters with grid (0.45  $\mu$  m pore size).
- 3.1.4. Membrane filtration unit (glass or stainless steel) consisting of individually packaged funnel and base.

##### 3.2. Non-sterile Equipment

- 3.2.1. 50 ml beaker, containing 20 ml of 95% ethanol for flaming forceps.
- 3.2.2. Blunt end forceps for handling membrane filters.
- 3.2.3. Polyethylene jar (2 liter) for used pipettes.

- 3.2.4. 2 liter, 2 outlet vacuum Erlenmeyer (waste collection) flask with vacuum tubing and one-way valve connected to the bottom outlet.
- 3.2.5. 1 liter, 2 outlet, plugged vacuum Erlenmeyer (water trap) flask connected with vacuum tubing, from the bottom outlet to the 2 liter flask and from the top outlet to the vacuum source.
- 3.2.6. Polyethylene bucket for wastewater (or to drain).
- 3.2.7. Bunsen burner.
- 3.2.8. Plastic "cakettes" for incubation of petri dishes.
- 3.2.9. Retort stand and base for holding membrane filter funnel when membrane filter is being removed from base (optional).
- 3.2.10. Pipetting device; eg. Pipet - Aid or other suitable device to avoid mouth pipetting.

### 3.3. Accessory Equipment

- 3.3.1. Waterbath and/or incubators for maintaining temperatures within  $\pm 0.5^{\circ}\text{C}$  in the ambient to  $60^{\circ}\text{C}$  range.
- 3.3.2. Stereoscopic microscope with 10X magnification for counting bacterial colonies.
- 3.3.3. Cool white fluorescent light for illuminating bacterial colonies.
- 3.3.4. Slanted ( $15^{\circ}$ ) dark-green wooden stage for resting petri dish during the counting operation.
- 3.3.5. Long-wave ultraviolet light source for detecting fluorescence.

## 4. Reagents

### 4.1. Sodium hydroxide solution (1 N)

In a 1 liter volumetric flask dissolve 40.0 g sodium hydroxide in distilled water and dilute to the mark.

### 4.2. Potassium dihydrogen orthophosphate solution (0.25 N)

In a 1 liter volumetric flask dissolve 34.02 g potassium dihydrogen orthophosphate in distilled water and dilute to the mark.

### 4.3. Buffered Water

To 1000 ml of distilled water add 1.25 ml of 0.25 M  $\text{KH}_2\text{PO}_4$  (previously adjusted to pH 7.2 with 1 N NaOH). The buffered water is made up in the containers listed below and sterilized in an autoclave.

- 4.3.1. 4 liter rinse bottle with dispensing nozzle.
- 4.3.2. 99 ml dilution blanks.
- 4.3.3. 90 ml dilution blanks.

### 4.4. Disinfectant for swabbing benches, disinfecting pipettes and filter effluent:

- 4.4.1. Wescodyne ("tamed iodine" - 1:30 with water)
- 4.4.2. Dettol (1:5 with alcohol or water)



#### **4.5. Bacteriological media and reagents:**

- 4.5.1. mPA agar
- 4.5.2. Skim Milk agar
- 4.5.3. Acetamide agar
- 4.5.4. Trypticase Soy agar (TSA)
- 4.5.5. Reagent for the Oxidase test  
- preparation of the above will be described fully in Section 7.
- 4.5.6. Reagents for the Gram Stain (see 8.6).

### **5. Procedure**

#### **5.1. General**

Samples must be kept refrigerated before analysis. Only samples which can be analyzed within one hour should be kept on the work bench. All others should be kept under refrigeration until they are required for analysis.

##### **5.1.1. Preparation for Membrane Filtration:**

- 5.1.1.1. Sterile technique must be employed throughout the analysis procedure.
- 5.1.1.2. The work bench area is swabbed thoroughly with Dettol solution and wiped dry.
- 5.1.1.3. Clean vacuum flasks and hoses are interconnected.
- 5.1.1.4. A small amount of Wescodyne, enough to immerse pipettes to zero mark, is poured into the waste bucket, pipette jar and waste collection flask.
- 5.1.1.5. The 4 liter rinse water bottle is set above the working area to allow for gravity flow. The dispensing hose and nozzle are hung using the retort stand and clamp above the water collection flask into which the membrane filter funnel and base will be placed. The hose may be fastened with tape to the clamp so that the flow of rinse water is not restricted and the glass nozzle does not come in contact with any other objects.
- 5.1.1.6. The samples are arranged in chronological order by laboratory number on the work bench, or on the basis of priority.
- 5.1.1.7. Petri dishes are laid out opposite each sample. Up to four filters may be used on each 100 mm square mPA plate. The plates should be inverted and the laboratory or sample number and dilutions are marked on the back of the plate.
- 5.1.1.8. The membrane filter unit (funnel and base) is unpacked and placed on the waste collection flask.

### 5.1.2. Preparation of Dilutions:

- 5.1.2.1. Samples for microbiological analysis frequently have large numbers of organisms and may require a series of one or more dilutions to provide a suitable distribution of colonies on the membrane filter before a satisfactory count can be made. Determination of the correct dilution series may be achieved by reference to previous analyses; knowledge of the nature of the samples; or on the observable turbidity of the sample and personal judgement.
- 5.1.2.2. Drinking water samples and relatively clean surface water samples usually require no dilution of the sample and aliquots from 100 ml to 1 ml are selected to give the appropriate distribution of organisms on the membrane filter.
- 5.1.2.3. Dilutions of samples are made up in buffered water dilution blanks just prior to the membrane filtration procedure. They should not be left standing on the bench for more than 5 minutes before filtration takes place.
- 5.1.2.4. The sample bottle is shaken 25 times vigorously.
- 5.1.2.5. The cap on the sample bottle is removed and held in one hand. The mouth of the bottle is flamed and one ml of sample is withdrawn with a pipette using a suitable pipetting device. The cap is screwed back into place.
- 5.1.2.6. The one ml aliquot is then dispensed into a 99 ml dilution blank. Before withdrawing aliquots for either further dilutions or filtration, the dilution blank must be shaken 25 times vigorously. If a ten ml aliquot is removed, this represents a 1:10 dilution of the original sample; if a one ml aliquot is removed, this is equivalent to 1:100 dilution. Higher dilutions are made by repeating the above operation. An alternative method of preparing a tenfold dilution series is the use of 90 ml dilution blanks and 10 ml pipettes for transferring 10 ml aliquots into each dilution blank.
- 5.1.2.7. Pipettes are used only once before discarding in the polyethylene jar for disposal or cleaning.

### 5.1.3. Membrane Filtration:

- 5.1.3.1. Prior to filtration of each sample or dilutions thereof, a control membrane is prepared. The vacuum supply of the membrane filter unit is turned on. The forceps are placed in the beaker containing 20 ml of 95% alcohol for several minutes; removed and flamed. Using the forceps, a membrane filter is removed from its package and held in one hand, while the membrane filter funnel is removed and held in the other hand. The membrane filter (grid side up) is then placed on the screen of the membrane filter base and the funnel is returned to the base and fastened securely. The forceps are returned to the alcohol beaker.
- 5.1.3.2. The funnel is then rinsed three times each with 20-30 ml of buffered water from the 4 liter rinse water bottle. The forceps are flamed again; the funnel removed and held; the

membrane filter carefully removed from the base with the forceps while the funnel is replaced on its base.

- 5.1.3.3. The free hand picks up the half of the petri dish containing the medium and, while holding it at an angle of  $45^{\circ}$ , the membrane filter is carefully rolled flat on one quadrant of the medium in the petri dish (grid side up). The petri dish is then inverted and placed back on its lid and the forceps are returned to the alcohol. Care must be exercised when placing the membrane filter on the medium so that no air bubbles are entrapped which would impede the diffusion of nutrients into the membrane filter. The forceps may be gently used around the edge of the membrane to remove entrapped air, but never in the area of the filtration.
- 5.1.3.4. Filtration of the respective portions of the sample either from dilution blanks or the original sample proceeds in the manner essentially as described above. Volumes of sample from 100 ml to 25 ml are measured out into a sterile graduated cylinder. Volumes of 10 ml to 1 ml are pipetted for filtration. Volumes less than 1 ml are not appropriate for analysis because of greater inaccuracies in pipetting small volumes. The first aliquot filtered is always the one containing the least amount of sample or the highest dilution of the sample.
- 5.1.3.5. Membrane filtration of an aliquot of sample proceeds as follows: a new membrane filter is positioned on the membrane filter base; the sample or dilution blank is shaken vigorously 25 times before measuring the volume for filtration; buffered rinse water is swirled into the funnel to a depth of 10-15 mm before and during dispensing of the aliquot in order to facilitate uniform distribution of cells on the membrane filters; the funnel is rinsed thoroughly 3 times each with 20-30 ml of buffered water; the membrane filter is removed from the unit and positioned on the growth medium in the petri dish. As before, sterile technique must be carefully observed; while handling the membrane filter with forceps; in the dispensing of the sample; and in putting the membrane filter on the growth medium. Any accidental contamination of the membrane filter, pipettes, graduated cylinders, bottle caps, etc. will require discarding of the contaminated object and the operation begun again.
- 5.1.3.6. Item 5.1.3.5. above is repeated for each aliquot of sample filtered. Items 5.1.3.1. - 5.1.3.5. are repeated for each sample.

#### 5.1.4 Incubation:

- 5.1.4.1 All petri dishes containing their respective membrane filters must be incubated within 30 minutes of the filtration step. The petri dishes are placed in separate plastic cakettes depending on the type of analysis, incubation period and incubation temperature. They are always incubated in an inverted position with the grid side of the membrane facing down. The bottom of the cakettes are lined with moistened paper towels to provide a humid atmosphere. Each cakette

is labelled to indicate the type of analysis, the date and time of incubation and the date and time of counting colonies on the membrane.

#### 5.1.5. Counting:

- 5.1.5.1. At the end of the incubation period, the bacterial colonies growing on the membranes are counted using a stereoscopic microscope with 10X magnification. Each petri dish is positioned on the slanted base and a cool white fluorescent light is set up to provide the best illumination.
- 5.1.5.2. Petri dishes should be observed and counts made within the designated incubation period. Prolonged incubation or standing of plates before observations are made could give inaccurate results with some types of colonies, as colour differentiation is required for identification of this particular group of microorganisms.

### 5.2. P. aeruginosa Enumeration

- 5.2.1. Water samples analyzed for numbers of P. aeruginosa by the membrane filter technique use a medium known as mPA agar. The mPA agar plates are incubated at  $41.5 \pm 0.5^{\circ}\text{C}$  for  $48 \pm 2$  hours. The plates are counted immediately after removal from the incubator using the stereoscopic microscope at a magnification of 10X.
- 5.2.2. Typical P. aeruginosa colonies are flat, 0.8 - 2.2 mm in diameter, and appear as tan to dark-brown or black. The colony usually has a dark centre and is lighter around the edges. Slowly developing P. aeruginosa colonies may be almost clear with just a small dark centre. P. aeruginosa colonies from chlorinated sewage effluents are often black, spreading colonies, and may be much larger than the typical P. aeruginosa colony. Background colonies usually appear red, pink, yellow or white. Doubtful colonies may be confirmed by streaking inoculum from the colony onto modified Skim Milk agar (8.4) with incubation at  $35^{\circ}\text{C}$  for 24 hours. A clearing of the agar due to caseinase production, and the production of a diffusable fluorescent pigment constitutes a positive result.
- 5.2.3. An ideal density range of colonies on a membrane filter consists of a total count not exceeding 150 colonies, and a P. aeruginosa count of 20 - 80 colonies. Plates with conditions unsuitable for counting are those with greater than 150 P. aeruginosa colonies, plates showing confluent growth of colonies, and plates with greater than 300 total colonies.

### 5.3. P. aeruginosa Isolation for Identification

#### 5.3.1. Purification:

- 5.3.1.1. Aseptically pick, with sterile inoculating needle, a single colony from the mPA filter and streak over a TSA plate.
- 5.3.1.2. Incubate TSA plate for 24 hrs. at  $35 \pm 0.5^{\circ}\text{C}$ .
- 5.3.1.3. Examine the TSA plate for colonial morphology. Typical P. aeruginosa colonies should be ovoid and flat, with a fuzzy edge and greenish pigment, although the pigment may vary, e.g. reddish, brown, etc.

- 5.3.1.4. Perform a Gram stain on a typical isolated P. aeruginosa colony.

5.3.2. **Gram Stain Procedure:**

- 5.3.2.1. Slides prepared from suspected P. aeruginosa colonies on TSA plates should first have one or two drops of water on the microscope slide. Using a flamed inoculating needle, a small portion of bacterial growth is removed from a colony. The inoculating needle is rubbed gently into the drop of water on the slide to allow a suspension of bacteria to form in the water. The inoculating needle is flamed and allowed to cool before using it to mix and spread the bacterial suspension into a thin film on the microscope slide. Precautions should be taken to avoid creating too dense a bacterial suspension, which could produce a slide which is difficult to read and interpret. The inoculating needle is flamed again.
- 5.3.2.2. If more than one bacterial suspension is prepared on one slide, a red wax marker should be used to separate the inoculated areas. All slide preparations are air-dried and heat-fixed before beginning the staining procedure. The slides are then taken over to a sink area and placed on a staining rack.
- 5.3.2.3. Crystal violet stain is applied to each slide and allowed to cover the area(s) on the slide which contain the bacterial growth. The crystal violet is left in contact with the bacterial cells for one minute. During the above staining period, the cold water tap is turned on to provide the minimum flow of water from the tap that can be obtained without changing to a series of drops. After one minute, each slide is placed in the stream of water about two inches from the orifice of the tap and the crystal violet is quickly washed from the slide.
- 5.3.2.4. The slide is placed back on the staining rack and the iodine solution is immediately added to the slide to cover the stained area(s) on the slide. The iodine solution is left in contact with the slide for a minimum period of one minute. If more than one slide is stained at the same time, the iodine solution may be left on the slide indefinitely until each other slide is decolourized and counterstained without any adverse effects.
- After one minute, the iodine solution is quickly, but gently washed from the slide and excess water is blotted off the slide with a paper towel. The slide is now ready for the decolourizing step.
- 5.3.2.5. After the excess water is blotted from the slide and before the slide has become dry, the 95% alcohol decolourizer is applied to the stained area(s) and the slide is tilted slightly back and forth to allow the alcohol to wash the stained area(s) on the slide. After 30 seconds, the alcohol is rinsed from the slide under the tap water in about 4 to 5 seconds.

5.3.2.6. The safranin counter stain is quickly added to the slide for a period of about one minute and then it is also quickly washed from the slide. Excess water is blotted from the slide with a paper towel and the slide is allowed to air-dry before making the microscopic examination.

5.3.2.7. The microscopic examination is done by placing the slide on the microscope stage positioning the slide so that the light is passing through one of the stained areas on the slide. With one of the low power objectives in place, the microscope is focused until the stained area on the slide is sharply in focus. The objective lens is moved out of position and a drop of immersion oil is placed in the center of the stained area. The immersion oil lens is moved into position and using the fine adjustment knob, the bacterial cells are carefully brought into focus.

If trouble is experienced in focusing the bacterial cells, the slide should be moved until one of the red wax markings on the slide is brought into view. The red wax marking on the surface of the slide is brought into sharp focus and the slide is carefully moved back towards the stained area of the slide while the viewer attempts to keep the surface of the slide in focus. Care must be taken that sufficient oil is present on the slide to allow this manipulation to take place.

5.3.2.8. For viewing and interpreting the Gram stain reaction of bacterial cells, the technician should endeavour to locate an area on the slide in which the bacterial cells are not clumped together but evenly dispersed. Bacterial cells which are deep purple or violet should be considered as Gram positive. Bacterial cells which are red or pink will be Gram negative. Difficulties in determining the Gram reaction of bacterial cells may be resolved somewhat if the light intensity is kept down to a minimum. If this does not work, a repeat Gram stain should be made on a 24-hour plate culture.

P. aeruginosa is a short Gram negative rod. Do not test mixed cultures further.

#### 5.4. P. aeruginosa Confirmation

##### 5.4.1. Skim Milk Agar:

5.4.1.1. Aseptically pick a typical, well isolated colony from the TSA plate and streak over a Skim Milk agar plate.

5.4.1.2. Incubate the Skim Milk agar plate for 24-48 hrs. at  $35 \pm 0.5^{\circ}\text{C}$ .

5.4.1.3. Pyocyanin production is evidenced by the production of a diffusible pigment, usually blue-green, that spreads through the medium.

5.4.1.4. Caseinase production is evidenced by a clearing of the agar around the colony caused by the hydrolysis of casein.

5.4.1.5. The production of a fluorescent pigment is also observable on Skim Milk agar. In a dark room, shine a long-wave



ultraviolet light source on the plate to see if a fluorescent pigment is present.

**NOTE:** P. aeruginosa will typically be caseinase positive, exhibit fluorescence and will produce a green diffusible pigment.

**5.4.2. Acetamide Utilization:**

5.4.2.1. Inoculate an Acetamide agar slant from the TSA agar or the Skim Milk agar and incubate at 35°C for 48 hours.

5.4.2.2. The utilization of acetamide will result in the release of ammonia and a reddening of the medium due to the resultant increase in pH; P. aeruginosa should show positive utilization of acetamide (8.5).

**5.4.3. Oxidase Production:**

5.4.3.1. Place a piece of Whatman no. 1 filter paper in a petri dish.

5.4.3.2. Moisten paper with two to three drops of tetramethyl reagent (see 7.5).

5.4.3.3. Using a platinum loop, remove a portion of a 24-hour culture from TSA or Skim Milk agar and smear onto the moistened filter paper.

5.4.3.4. A positive reaction is indicated by a lavender to purple black colour development within 15 seconds.

**6. Calculation and Reporting**

The P. aeruginosa and background densities are recorded in terms of number of organisms per 100 ml. The general equation for calculating results would be:

$$\text{Number of organisms per 100 ml} = \frac{100 \times \text{colonies counted}}{\text{ml sample filtered.}}$$

**7. Preparation of Media for P. aeruginosa Analysis**

**7.1. Medium for P. aeruginosa enumeration**

**7.1.1. Apparatus for the Preparation of mPA Medium**

7.1.1.1. Stirring hot plates (2)

7.1.1.2. Glass beaker (or other suitable container) 4 liter

7.1.1.3. Glass beakers 500 ml (2), sterile

7.1.1.4. Stirring magnets (2)

7.1.1.5. Asbestos gloves

7.1.1.6. Analytical balance, sensitive to 0.0001 g

7.1.1.7. Top loading balance, sensitive to 0.01 g

7.1.1.8. pH meter

7.1.1.9. Thermometer (0-110°C)

7.1.1.10. Aluminum foil

7.1.1.11. Graduated cylinder 1 liter

- 7.1.1.12. Spatula
- 7.1.1.13. Disposable petri dishes, sterile, 100 x 15 mm square
- 7.1.1.14. Plastic "cakettes"

**7.1.2. Reagents Required for the Preparation of mPA Plates**

- 7.1.2.1. L-Lysine monohydrochloride - BDH
- 7.1.2.2. Yeast extract - Oxoid
- 7.1.2.3. Xylose - Difco
- 7.1.2.4. Sodium thiosulphate ( $\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$ ), reagent grade
- 7.1.2.5. Sucrose (Difco)
- 7.1.2.6. Lactose (Difco)
- 7.1.2.7. Phenol red indicator (Matheson, Coleman and Bell)
- 7.1.2.8. Ferric ammonium citrate (J.T. Baker)
- 7.1.2.9. Sodium chloride (NaCl) reagent grade
- 7.1.2.10. Agar (Difco)
- 7.1.2.11. Distilled water
- 7.1.2.12. Sulfapyridine (Sigma)
- 7.1.2.13. Kanamycin sulphate (Sigma)
- 7.1.2.14. Naladixic acid (Winthrop)
- 7.1.2.15. Actidione (Upjohn)
- 7.1.2.16. Magnesium sulphate (reagent grade)
- 7.1.2.17. Sodium desoxycholate (reagent grade)
- 7.1.2.18. Sodium hydroxide (NaOH) reagent grade pellets
- 7.1.2.19. Sodium hydroxide Solution (1 N)

In a 1 liter volumetric flask dissolve 40 g sodium hydroxide in distilled water and dilute to volume

**7.1.3. Preparation of 1 liter of mPA:**

- 7.1.3.1. Measure out 800 ml of distilled water into the 4 liter glass beaker. Place the stirring magnet in the beaker and put it on a stirring hot plate, activating the stirring mechanism to medium speed.
- 7.1.3.2. Check the top loading balance level and zero the balance. Place the weighing dish on the balance and tare the balance to zero.
- 7.1.3.3. Weigh out individually the following ingredients and dissolve consecutively in the distilled water:
  - 7.1.3.3.1. L-Lysine monohydrochloride - 5.0 g
  - 7.1.3.3.2. Yeast extract - 2.0 g
  - 7.1.3.3.3. Xylose - 2.5 g
  - 7.1.3.3.4. Sodium thiosulphate - 5.0 g



- 7.1.3.3.5. Sucrose - 1.25 g
- 7.1.3.3.6. Lactose - 1.25 g
- 7.1.3.3.7. Phenol red - 0.08 g (use analytical balance)
- 7.1.3.3.8. Ferric ammonium citrate - 0.8 g
- 7.1.3.3.9. Sodium desoxycholate - 0.2 g
- 7.1.3.3.10. Magnesium sulphate - 1.5 g
- 7.1.3.3.11. Sodium chloride - 5.0 g
- 7.1.3.4. Adjust pH to 7.35 with 1N sodium hydroxide solution. Weigh out 15.0 g of agar and add slowly to the beaker. The mouth of the beaker is then covered with aluminum foil and a thermometer is inserted through the aluminum foil. Heat is applied to the beaker until the medium starts to boil or the agar shows signs of being in solution about 90-92°C.
- 7.1.3.5. Allow to cool slowly to 55 - 60°C, while preparing the antibiotic solution. Stir continuously.
- 7.1.3.6. Check the analytical balance level and zero the balance. Place the weighing dish or aluminum foil on the balance and tare the balance to zero.
- 7.1.3.7. Weigh out individually the following antibiotics and dissolve consecutively in 200 ml of sterile distilled water in a 500 ml sterile beaker.
  - 7.1.3.7.1. Sulfapyridine - 88 mg
  - 7.1.3.7.2. Kanamycin sulphate - 8.5 mg
  - 7.1.3.7.3. Naladixic acid - 37 mg
  - 7.1.3.7.4. Actidione - 150 mg
- 7.1.3.8. Heat to 55 - 60°C and stir the mixture until all antibiotics are dissolved.
- 7.1.3.9. When both the antibiotic solution and agar portion of the medium are at 55 - 60°C, they are mixed together and stirred thoroughly. A small amount of the mixture is poured into a petri dish and allowed to solidify. The pH of the surface of the medium should be 7.1. If necessary, 1 N sodium hydroxide may be used to adjust the pH of the medium before pouring into petri dishes.
- 7.1.3.10. Pour approximately 350 ml into a 500 ml beaker and dispense into petri dishes in a laminar flow hood. The lids are left slightly open to permit cooling of the medium without excessive condensation on the lids of the petri dishes. Repeat until all the medium is dispensed.
- 7.1.3.11. After the plates have cooled and the medium has solidified, the plates are inverted and stored in plastic cakettes in a refrigerator. The final pH of the medium should be  $7.1 \pm 0.1$ .

## **7.2. Medium for P. aeruginosa Isolation and Purification**

This is a general purpose medium used for growing bacterial cultures for inoculating other microbiological tests or for storage of cultures for 3-4 weeks.

### **7.2.1. Apparatus for the Preparation of Trypticase Soy Agar**

- 7.2.1.1. Stirring hot plate
- 7.2.1.2. Stainless steel beaker, 2 liter
- 7.2.1.3. Stirring magnet
- 7.2.1.4. Asbestos gloves
- 7.2.1.5. Top loading balance, sensitive to 0.01 g
- 7.2.1.6. Spatula
- 7.2.1.7. Automatic dispenser set at 5.0 ml
- 7.2.1.8. Graduated cylinder, 1 or 2 liter
- 7.2.1.9. Weighing dish
- 7.2.1.10. Thermometer (0-110°C)
- 7.2.1.11. Aluminum foil
- 7.2.1.12. Disposable petri plates, sterile 100 x 15 mm, round, (50)
- 7.2.1.13. Glass beaker 500 ml
- 7.2.1.14. Plastic "cakettes"

### **7.2.2. Reagents Required for the Preparation of Trypticase Soy Agar**

- 7.2.2.1. Tryptic Soy agar (Difco)
- 7.2.2.2. Distilled water

### **7.2.3. Preparation of Trypticase Soy Agar**

- 7.2.3.1. Measure out 1 liter of distilled water and pour into the 2 liter stainless steel beaker.
- 7.2.3.2. Place a stirring magnet (alcohol flamed) in the beaker and put the beaker on the stirring hot plate. Activate the stirring mechanism to medium speed.
- 7.2.3.3. Check balance level and zero the balance. Place the weighing dish on the balance and tare the balance to zero.
- 7.2.3.4. Weigh out 40.0 g of the Trypticase Soy agar powder using a clean spatula.
- 7.2.3.5. Slowly pour the powder medium into the beaker of distilled water. Continue the stirring action and turn on the heating element. Cover the mouth of the beaker with aluminum foil and insert a thermometer through the foil at the side of the beaker. Heat the medium to a slow boil for one minute or until the agar has gone into solution, 90-92°C.
- 7.2.3.6. Remove the thermometer, cover hole with autoclave tape, and autoclave solution for 15 minutes at 121°C.
- 7.2.3.7. Allow medium to cool to approximately 50°C and then pour about 350 ml into the 500 ml beaker and dispense this

medium into plates. Repeat until all the medium has been dispensed. Allow plates to cool and solidify with the lids partly removed to prevent excessive condensation. Store the plates, inverted, in plastic cakettes in a refrigerator. The final surface pH of the medium should be  $7.3 \pm 0.2$ .

### 7.3. Skim Milk Agar for P. aeruginosa Confirmation

Skim Milk agar is used to test for pigment production and caseinase activity.

#### 7.3.1. Apparatus for Preparation of Skim Milk Agar

- 7.3.1.1. Stirring hot plates (2)
- 7.3.1.2. Stainless steel beaker 2 liter
- 7.3.1.3. Stainless steel beaker 1 liter
- 7.3.1.4. Stirring magnets (2)
- 7.3.1.5. Asbestos gloves
- 7.3.1.6. Top loading balance, sensitive to 0.01 g
- 7.3.1.7. Spatula
- 7.3.1.8. Graduated cylinder, 1 liter
- 7.3.1.9. Weighing dish
- 7.3.1.10. Thermometer (0-110°C)
- 7.3.1.11. Petri dishes, 100 x 15 mm round or square plastic (40)
- 7.3.1.12. Plastic cakettes with lids
- 7.3.1.13. Aluminum foil
- 7.3.1.14. Electric fan
- 7.3.1.15. Sterile 500 ml glass beaker
- 7.3.1.16. 5% Wescodyne

#### 7.3.2. Reagents Required for the Preparation of Skim Milk Agar

- 7.3.2.1. Dehydrated Skim Milk powder
- 7.3.2.2. Agar (Difco)
- 7.3.2.3. Distilled water

#### 7.3.3. Preparation of 1 liter of Skim Milk Agar:

- 7.3.3.1. Measure out 500 ml of distilled water into the 2 liter beaker. Place a stirring magnet (alcohol flamed) in the beaker and put it on a stirring plate which has the stirring mechanism set for medium speed.
- 7.3.3.2. Check the balance level and zero the balance. Place a weighing dish on the balance and tare the balance to zero.
- 7.3.3.3. Weigh out 100 g of Skim Milk powder and add it slowly to the 500 ml of distilled water. Allow the mixture to stir without heat for approximately 30 minutes.
- 7.3.3.4. During this period, measure out 500 ml of distilled water into the 1 liter beaker. Place a stirring magnet (alcohol flamed) in the beaker and put on a stirring hot plate, which has the stirring mechanism set at medium speed.

- 7.3.3.5. Using a spatula, weigh out 15.0 g of agar and slowly add the agar to the 500 ml of distilled water. Turn on the heating element, cover the beaker mouth with aluminum foil, place a thermometer on the inside of the beaker, and allow the temperature of the medium to slowly rise to 90-92°C. This usually takes 10-12 minutes.
- 7.3.3.6. Both the beaker containing the dissolved agar and the beaker containing the dissolved Skim Milk are covered with aluminum foil and placed in the autoclave for sterilization at 121°C for 12 minutes. Both beakers are removed from the autoclave immediately and placed on stirring plates until the temperature of each has cooled to approximately 55°C.
- 7.3.3.7. At the start of the cooling period, the laminar flow hood is swabbed with 5% Wescodyne and the petri dishes are laid in four to five rows.
- 7.3.3.8. The Skim Milk solution is then aseptically added in the agar solution and the mixture is stirred for an additional two to three minutes until the temperature has dropped to 50-52°C.
- 7.3.3.9. When the temperature has cooled sufficiently for pouring plates, the sterile 500 ml beaker is three-quarters filled with the Skim Milk medium. This beaker is used for pouring about 20-25 ml of medium into each petri dish. The lids are left slightly open to permit cooling of the medium without excess condensation on the lids of the petri dish. When the medium has solidified, the lids are closed and the petri dishes are inverted. They are then placed in plastic cakettes into the refrigerator for use within two weeks. The final pH of the medium is  $6.4 \pm 0.2$ .

#### 7.4. **Acetamide Agar for P. aeruginosa Confirmation**

This medium is used to demonstrate the presence of P. aeruginosa.

##### 7.4.1. **Apparatus for the Preparation of Acetamide Agar Slants**

- 7.4.1.1. Stirring hot plate
- 7.4.1.2. Stainless steel beaker 2 liter
- 7.4.1.3. Stirring magnet
- 7.4.1.4. Asbestos gloves
- 7.4.1.5. Top loading balance, sensitive to 0.01 g
- 7.4.1.6. Spatula
- 7.4.1.7. Automatic dispenser set at 10 ml
- 7.4.1.8. Screw cap tubes 20 x 125 mm (100 tubes)
- 7.4.1.9. Rubber lined, bakelite screw caps 20 mm size (100 caps)
- 7.4.1.10. Test tube baskets
- 7.4.1.11. Slanting racks
- 7.4.1.12. Analytical balance, sensitive to 0.0001 g
- 7.4.1.13. pH meter

- 7.4.1.14. Thermometer (0-110°C)
- 7.4.1.15. Aluminum foil
- 7.4.1.16. Graduated cylinder, 1 liter
- 7.4.2. **Reagents Required for the Preparation of Acetamide Agar**
  - 7.4.2.1. Sodium chloride (NaCl) - BDH
  - 7.4.2.2. Di-potassium hydrogen phosphate ( $K_2HPO_4$ ) reagent grade
  - 7.4.2.3. Acetamide ( $CH_3CONH_2$ ) reagent grade
  - 7.4.2.4. Potassium dihydrogen phosphate ( $KH_2PO_4$ ) reagent grade
  - 7.4.2.5. Magnesium sulphate ( $MgSO_4 \cdot 7H_2O$ ) reagent grade
  - 7.4.2.6. Phenol red indicator reagent grade (Matheson, Coleman, and Bell)
  - 7.4.2.7. Agar - Difco
  - 7.4.2.8. Distilled water
  - 7.4.2.9. Sodium hydroxide 0.2 N
  - 7.4.2.10. Hydrochloric acid 0.2 N
- 7.4.3. **Preparation of 1 liter Acetamide Agar Slants:**
  - 7.4.3.1. Measure out 1 liter of distilled water into the 2 liter stainless steel beaker. Place the stirring magnet in the beaker and put it on a stirring hot plate, activating the stirring mechanism to medium speed.
  - 7.4.3.2. Check the balance level and zero the balance. Place the weighing dish on the balance and tare the balance to zero.
  - 7.4.3.3. Weigh out individually the following ingredients and dissolve consecutively in the distilled water:
    - 7.4.3.3.1. Sodium chloride - 5.0 g
    - 7.4.3.3.2. Di-potassium hydrogen phosphate - 1.4 g
    - 7.4.3.3.3. Acetamide - 10.0 g
    - 7.4.3.3.4. Potassium dihydrogen phosphate - 0.7 g
    - 7.4.3.3.5. Magnesium sulphate - 1.0 g
    - 7.4.3.3.6. Phenol red indicator - 0.012 g (use analytical balance)
  - 7.4.3.4. When the ingredients are in solution, the pH should be checked and adjusted to pH 6.8 with 0.2 N NaOH or HCl as required.
  - 7.4.3.5. Weigh out 15.0 g of agar and add slowly to the beaker. The mouth of the beaker is then covered with aluminum foil and a thermometer is inserted through the aluminum foil. Heat is applied to the beaker until the medium starts to boil or the agar shows signs of being in solution about 90-92°C.
  - 7.4.3.6. The medium is then dispensed in 5.0 ml volumes into the tubes; the tubes are loosely capped; and the medium is autoclaved for 15 minutes at 121°C.

- 7.4.3.7. The baskets of tubes are removed from the autoclave at the end of the cycle and the tubes are placed individually on slanting racks which are slanted to provide a butt about 20 mm deep.
- 7.4.3.8. When the medium has cooled and solidified, the caps are tightened and the medium is placed in the refrigerator for a period not exceeding 3 months. The final pH of the medium is  $6.8 \pm 0.2$ .

## 7.5. Oxidase Reagent for P. aeruginosa Confirmation

Used to determine if the organism under examination is oxidase positive.

### 7.5.1. Apparatus for Preparation of Oxidase Reagent

- 7.5.1.1. Graduated cylinder, 100 ml
- 7.5.1.2. Beaker, 250 ml
- 7.5.1.3. Dark glass stoppered bottle, 150 ml, sterile
- 7.5.1.4. Hot plate
- 7.5.1.5. Spatula
- 7.5.1.6. Top loading balance, sensitive to 0.01 g

### 7.5.2. Reagents Required for the Preparation of Oxidase Reagent

- 7.5.2.1. N,N,N',N'-tetramethyl-p-phenylenediamine dihydrochloride (J.T. Baker)
- 7.5.2.2. L-ascorbic acid (BDH)
- 7.5.2.3. Sterile distilled water

### 7.5.3. Preparation of 100 ml of oxidase reagent:

- 7.5.3.1. Measure out 100 ml of distilled water into sterile beaker.
- 7.5.3.2. Add 0.1 g of ascorbic acid to the distilled water.
- 7.5.3.3. Add 1.0 g of N,N,N',N'-tetramethyl-p-phenylenediamine dihydrochloride to the ascorbic acid solution, and dissolve it with gentle warming if necessary (no higher than 35°C). Store in dark glass-stoppered bottle at 4°C.
- 7.5.3.4. Before using, allow the solution to stand at room temperature for 15 minutes.

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## THE ENUMERATION OF TOTAL COLIFORMS, FECAL COLIFORMS AND FECAL STREPTOCOCCI

Pollution indicator bacteria are used to determine the potability or potential health hazard associated with a water supply, bathing area or a wastewater effluent. Particular groups of pollution indicator bacteria were chosen because of their association with fecal material, which must always be considered as a potential source of disease-producing organisms. Under most conditions, these bacteria are present in numbers much greater than any disease-producing organisms and in general are considered to survive longer than pathogenic microorganisms. In addition, techniques for direct isolation and enumeration of pathogenic organisms have so far had limited success or acceptance by public health agencies.

Analyses for the detection and quantification of pollution in samples are most frequently performed for total coliforms, fecal coliforms and fecal streptococci. The interpretation and significance of these bacterial groups in water depends on the types and numbers of the organisms and the intended use of the water (8.5.).

### Sample Handling and Preservation

Bacteriological samples must be collected in Ministry of the Environment (MOE) sterilized glass or plastic bottles. The bacteriological examination of a water sample should be initiated immediately after collection. However, as this is seldom practical, more realistic arrangements must be established. The sample bottles should be chilled (not frozen) and transported to the laboratory within 24 hours. In any event, bacteriological analyses are not done on samples aged three days or more. For specific programs, samples must arrive at the laboratory within 24 hours. Samples containing chlorine as a disinfecting agent should be collected in sterile bottles to which sodium thiosulphate has been added to neutralize any chlorine present.

### Selection of Method

The two procedures commonly used to detect and quantify pollution indicator bacteria are the Most Probable Number (MPN) method and the Membrane Filter (MF) method. Each method has its own advantages and disadvantages, but for the most part they are considered to give comparable results. From a statistical aspect, the MF method gives direct counts; better reproducibility of results; produces results in 24-48 hours, and allows the examination of larger volumes of sample than the MPN method. The examination of water samples with high turbidity due to algae or other material may not permit testing of sufficient sample volume to give significant results by the MF method. Similarly, samples with low numbers of indicator organisms or the presence of interfering substances may require the use of the MPN procedure to obtain accurate results.



## TOTAL COLIFORM AND BACKGROUND COUNTS

### MEMBRANE FILTER ANALYSIS

#### SUMMARY

<b>Matrix.</b>	This method is used on drinking water, surface water, sewage and industrial waste samples.
<b>Organisms determined</b>	The test is designed to measure total coliform and non-coliform or background bacteria present in water samples from a variety of sources.
<b>Interpretation of results</b>	In drinking water samples, the presence of total coliform bacteria indicates inadequate treatment of the water. Background bacteria represent non-coliform bacteria, which if present in large numbers may indicate poor quality water; they may also interfere with the determination of total coliforms.
<b>Principle of method</b>	Each bacterial cell deposited on the membrane filter and given a suitable nutrient source has the potential to grow and multiply sufficiently to form a bacterial colony with distinguishing characteristics to permit a differential count being made.
<b>Time required for analysis</b>	Depending on the density of bacteria in the sample, and hence the number of dilutions required to obtain plates suitable for counting, the analysis time for one sample would range from 5 - 10 minutes. Incubation time for the total coliforms is $22 \pm 2$ hours.
<b>Range of application</b>	Maximum sample volume for which an analysis is usually done is 100 ml. The upper limit of bacteria determined is dependent only on the number of dilutions required to obtain a suitable count.
<b>Standard deviation</b>	Performance characteristics are not yet available.
<b>Accuracy</b>	Performance characteristics are not yet available.
<b>Detection criteria</b>	Performance characteristics are not yet available.
<b>Interferences and shortcomings</b>	Improper choice of dilutions for membrane filters will affect the ability to obtain plates suitable for counting. High populations of non-coliform bacteria and toxic substances in the sample or membrane filters will interfere with colony and sheen development of coliform bacteria.

**Minimum volume  
of sample**

200 ml of sample is preferred when tests for several groups of bacteria are requested.

**Preservation and  
sample container**

MOE 250 ml sterile glass bottles should be used for collection of samples for drinking water analyses. Those from distribution systems should contain sodium thiosulphate to neutralize any chlorine residual. Samples should be iced if possible during transportation to the laboratory and arrive within 24 hours of the sampling time.

**Safety  
considerations**

Bacteriological samples, particularly those from sources of water suspected of pollution, may contain pathogenic organisms, and should be handled in a manner to prevent contamination of the sampler and the analyst.

## FECAL COLIFORM AND FECAL STREPTOCOCCUS COUNTS

### MEMBRANE FILTER ANALYSIS

#### SUMMARY

Matrix.	This method is used to determine fecal pollution in water samples.
Organisms determined	The fecal coliform and fecal streptococci tests are frequently done together to determine any interrelationship (8.3.).
Interpretation of results	Fecal coliforms and fecal streptococci should be absent from potable drinking water samples. In natural waters, use is made of fecal coliform (FC) to fecal streptococci (FS) ratios of counts to determine the nature of the pollution. FC:FS ratios greater than 4.0 suggest human fecal pollution whereas FC:FS ratios less than 0.7 suggest animal fecal pollution. Intermediate ratios suggest a mixture of human and animal fecal pollution, but they may only indicate a more rapid decrease in viability of one population of organisms over another. The FC:FS ratio can only be used when both indicators suggest polluted conditions (FC > 100/100 ml or FS > 100/100 ml) and is applicable only to results from areas in the immediate vicinity of the waste effluent discharge.
Principle of method	Each bacterial cell deposited on the membrane filter and given a suitable nutrient source has the potential to grow and multiply sufficiently to form a bacterial colony with distinguishing characteristics to permit a differential count being made.
Time required for analysis	Depending on the density of bacteria in the sample, and hence the number of dilutions required to obtain plates suitable for counting, the analysis time for one sample would be about five minutes for each parameter. Incubation time for fecal coliforms is $23 \pm 1$ hour and for fecal streptococci, it is $48 \pm 3$ hours.
Range of application	Maximum sample volume for which an analysis is usually done is 100 ml. The minimum bacterial count is one organism per 100 ml. The upper limit of bacteria determined is dependent only on the number of dilutions required to obtain a suitable count.
Standard deviation	Performance characteristics are not yet available.
Accuracy	Performance characteristics are not yet available.
Detection criteria	Performance characteristics are not yet available.

**Interferences  
and Shortcomings**

Improper choice of dilutions for membrane filters will affect the ability to obtain plates suitable for counting. High populations of organisms, inhibitory factors associated with membrane filters and/or toxic substances in the samples will interfere with the development of the organism for which the test was designed.

**Minimum volume  
of sample**

200 ml of sample is preferred when tests for several groups of bacteria are requested.

**Preservation and  
sample container**

MOE 250 ml sterile glass bottles or sterile 250 ml Nalgene bottles may be used for sample collection. Sample bottles for collection of chlorinated waters should have sodium thiosulphate added. All samples should be iced during transportation to the laboratory and preferably arrive for analysis within 24 hours of sampling time.

**Safety  
considerations**

Bacteriological samples, particularly those from sources of water suspected of pollution, may contain pathogenic organisms, and should be handled in a manner to prevent contamination of the sampler and the analyst.

**TOTAL COLIFORM, FECAL COLIFORM  
AND FECAL STREPTOCOCCUS  
MEMBRANE FILTER ANALYSIS**

**1. Introduction**

The determination of total coliforms, fecal coliforms and fecal streptococci by membrane filtration employs similar procedures, differing only in the growth medium, incubation temperature and time, and the differentiation of colonies on MF petri plates. A general description of the membrane filter procedure will be given first and this will be followed by individual descriptions of salient factors to be observed for the analysis of total coliforms, fecal coliforms and fecal streptococci.

**2. Interferences and Shortcomings**

The enumeration of each of the indicator bacteria depends on the selection of an appropriate volume of sample for testing purposes. In some instances, the sample volume may be too small to detect any indicator organisms. Conversely, if the sample volume is too large, an overgrowth or overabundance of bacterial colonies may interfere with obtaining an accurate count of the respective indicator group.

For certain samples, bacterial cells may have been exposed to adverse environmental factors which lower their probability for survival and growth on a membrane filter medium. Similarly, certain membranes interfere with the growth of bacterial colonies (8.1., 8.2.).

**3. Apparatus**

**3.1. Sterile Equipment**

- 3.1.1. Pipettes, 1 or 2 ml for 1 ml deliveries, 10 ml for 5 and 10 ml deliveries.
- 3.1.2. Graduate cylinders, 100 ml for 100 ml volumes, 50 ml for 50 ml and 25 ml volumes.
- 3.1.3. Disposable petri dishes, round 50 x 12 mm, square 100 x 15 mm.
- 3.1.4. Membrane filters, 47 mm with grid (0.45  $\mu$ m pore size).
- 3.1.5. Membrane filtration unit (glass or stainless steel) consisting of individually packaged funnel and base, or the unit may be stored within a sterile container.

**3.2. Non-sterile Equipment**

- 3.2.1. Beaker, 50 ml, containing 20 ml of 95% ethanol for flaming forceps.

- 3.2.2. Forceps, blunt-end for handling membrane filters.
- 3.2.3. Polyethylene jar (2 liter) for used pipettes.
- 3.2.4. Vacuum Erlenmeyer (waste collection) flask, 2 outlet, 2 liter, with vacuum tubing and one-way valve connected to the bottom outlet.
- 3.2.5. Vacuum Erlenmeyer (water trap) flask, plugged, 2 outlet, 1 liter, connected with vacuum tubing, from the bottom outlet to the 2 liter flask and from the top outlet to the vacuum source.
- 3.2.6. Polyethylene bucket for wastewater (or to drain).
- 3.2.7. Bunsen burner.
- 3.2.8. Plastic "cakettes" for incubation of petri dishes.
- 3.2.9. Retort stand and clamp for holding membrane filter funnel when membrane filter is being removed from base. (optional).
- 3.2.10. Pharal jars, opaque polystyrene jars (7.5 cm tall and 6.5 cm diameter) with white plastic screw cap lids.
- 3.2.11. Pipetting device; eg. Pipet - Acid or other suitable device to avoid mouth pipetting.

### 3.3. Accessory Equipment

- 3.3.1. Waterbath and/or incubators for maintaining temperatures within  $\pm 0.5^{\circ}\text{C}$  in the ambient to  $60^{\circ}\text{C}$  range.
- 3.3.2. Stereoscopic microscope with 10X magnification for counting bacterial colonies.
- 3.3.3. Cool white fluorescent light for illuminating bacterial colonies.
- 3.3.4. Slanted ( $15^{\circ}$ ) wooden stage for resting petri dish during the counting operation.

## 4. Reagents

- 4.1. Potassium dihydrogen orthophosphate ( $\text{KH}_2\text{PO}_4$ ), reagent grade powder.
- 4.2. Sodium hydroxide ( $\text{NaOH}$ ) reagent grade pellets.
- 4.3. Potassium Dihydrogen Orthophosphate Solution (0.25 M).

In a 1 liter volumetric flask dissolve 34.0 g potassium dihydrogen orthophosphate in distilled water and dilute to mark.

- 4.4. Sodium Hydroxide Solution (1N)

In a 1 liter volumetric flask dissolve 40.0 g sodium hydroxide in distilled water and dilute to the mark.

#### 4.5. Buffered Water

To 1000 ml distilled water add 1.25 ml of 0.25 M potassium dihydrogen phosphate solution (previously adjusted to pH 7.2 with 1N sodium hydroxide solution). Make up solution in containers listed below and sterilize in an autoclave.

4.5.1. 4 liter rinse bottle with dispensing nozzle.

4.5.2. 99 ml dilution blanks.

4.5.3. 90 ml dilution blanks.

#### 4.6. Disinfectant for swabbing benches, disinfecting pipettes and filter effluent:

4.6.1. Wescodyne ("tamed iodine" 1:30 with water).

4.6.2. Dettol (1:5 with water).

#### 4.7. Bacteriological media:

4.7.1. Total Coliforms - Bacto m-Endo agar LES (Difco).

4.7.2. Fecal Coliforms - m-TEC agar.

4.7.3. Fecal Streptococci - Bacto m-Enterococcus agar (Difco)

- preparation of the above media will be described fully in section (7).

### 5. Procedure

#### 5.1. General

5.1.1. Samples must be kept refrigerated before analysis. Only samples which can be analyzed within one hour should be kept on the work bench. All others should be kept under refrigeration until they are required for analysis.

#### 5.1.2. Preparation for Membrane Filtration:

5.1.2.1. Sterile technique must be employed throughout the analysis procedure.

5.1.2.2. The work bench area is swabbed thoroughly with the Dettol solution and wiped dry.

5.1.2.3. Clean vacuum flasks and hoses are interconnected.

5.1.2.4. A small amount of Wescodyne is poured into the waste bucket, pipette jar and waste collection flask.

5.1.2.5. The 4 liter rinse water bottle is set above the working area to allow for gravity flow. The dispensing hose and nozzle are hung using the retort stand and clamp, above the water collection flask into which the membrane filter funnel and base will be placed. The hose may be fastened with tape to the clamp so that the flow of rinse water is not restricted

and the glass nozzle does not come in contact with any other objects.

- 5.1.2.6. The samples are arranged in order by laboratory number on the work bench or on the basis of priority.
- 5.1.2.7. Petri dishes are laid out opposite each sample. Those containing Endo agar LES, m-TEC agar and Enterococcus agar are inverted and the laboratory number and dilution are marked on the back of each plate.
- 5.1.2.8. The membrane filter unit (funnel and base) is unpacked and placed on the waste collection flask.

### 5.1.3. Preparation of Dilutions:

- 5.1.3.1. Samples for microbiological analysis frequently have large numbers of organisms and require a series of one or more dilutions to provide a suitable distribution of colonies on the membrane filter before a satisfactory count can be made. Determination of the correct dilution series may be achieved by reference to previous analyses; knowledge of the nature of the sample; or sometimes based only on the observable turbidity of the sample and personal judgement.
- 5.1.3.2. Drinking water samples and relatively clean surface water samples usually require no dilution of the sample and aliquots from 100 ml to 1 ml are selected to give the appropriate distribution of organisms on the membrane filter. Samples from rivers, sewage effluents and other polluted sources frequently require one or more dilutions of the original sample (ten-fold dilution series is preferred.)
- 5.1.3.3. Dilutions of samples are made up in buffered water dilution blanks just prior to the membrane filtration procedure. They should not be left standing on the bench for more than 5 minutes before filtration takes place.
- 5.1.3.4. The sample bottle is shaken 25 times vigorously.
- 5.1.3.5. The cap on the sample bottle is removed and held in one hand. The mouth of the bottle is flamed and one ml of sample is withdrawn with a pipette using a suitable pipetting device. The mouth of the sample bottle is flamed again and the cap screwed back into place.
- 5.1.3.6. The one ml aliquot is then dispensed into a 99 ml dilution blank. Before withdrawing aliquots for either further dilutions or filtration, the dilution blank must be shaken 25 times vigorously. If a ten ml aliquot is removed, this represents a 1:10 dilution of the original sample; if a one ml aliquot is removed, this is equivalent to 1:100 dilution. Higher dilutions are made by repeating the above operation. An alternative method of preparing a ten-fold dilution series may be done using 90 ml dilution blanks and 10 ml pipettes for transferring 10 ml aliquots into each dilution blank.
- 5.1.3.7. Pipettes are used only once before discarding in the polyethylene jar.



#### 5.1.4. Membrane Filtration:

- 5.1.4.1. Prior to filtration of each sample or dilutions thereof, a control membrane is prepared. The vacuum supply of the membrane filter unit is turned on. The forceps are placed in the beaker containing 20 ml of 95% alcohol for several minutes; removed and flamed. Using the forceps, a membrane filter is removed from its package and held in one hand, while the membrane filter funnel is removed and held in the other hand. The membrane filter (grid side up) is then placed on the screen of the membrane filter base and the funnel is returned to the base and fastened securely. The forceps are returned to the alcohol beaker.
- 5.1.4.2. The funnel is then rinsed three times each with 20-30 ml of buffered water from the 4 liter rinse water bottle. The forceps are flamed again; the funnel removed and held; the membrane filter carefully removed from the base with the forceps while the funnel is replaced on its base.
- 5.1.4.3. The other hand picks up the half of the petri dish containing the medium and while holding it at an angle of 45°, the membrane filter is carefully rolled flat on one quadrant of the medium in the petri dish (grid side up). The petri dish is then inverted and placed back on its lid and the forceps are returned to the alcohol. Care must be exercised when placing the membrane filter onto the medium so that no air bubbles are entrapped which would impede the diffusion of nutrients into the membrane filter. The forceps may be gently used around the edge of the membrane to remove entrapped air, but never in the area of the filtration.
- 5.1.4.4. Filtration of the respective portions of the sample either from dilution blanks or the original sample proceeds in a manner essentially as described above. Volumes of sample from 100 ml to 25 ml are measured out into a sterile graduate cylinder. Volumes of 10 ml to 1 ml are pipetted for filtration. Volumes of less than 1 ml are not appropriate for analysis because of inaccuracies in pipetting small volumes. The first aliquot filtered is always the one containing the least amount of sample or the highest dilution of the sample.
- 5.1.4.5. Membrane filtration of an aliquot of sample proceeds as follows: a new membrane filter is positioned on the membrane filter base; the sample or dilution blank is shaken vigorously 25 times before measuring the volume for filtration; buffered rinse water is swirled into the funnel to a depth of 10-15 mm before and during dispensing of the aliquot (facilitates uniform distribution of cells on the membrane filter); the funnel is rinsed thoroughly 3 times each with 20-30 ml of buffered water; the membrane filter is removed from the unit and positioned on the growth medium in the petri dish. As before, sterile technique in handling the membrane filter with the forceps, dispensing the sample, and putting the membrane filter on the growth medium, must be carefully observed. Any accidental

contamination of the membrane filter, pipettes, graduate cylinders, bottle caps, etc. will require discarding of the contaminated object and the operation begun again.

- 5.1.4.6. Item 5.1.4.5. above is repeated for each aliquot of sample filtered. Items 5.1.4.1. to 5.1.4.5. are repeated for each sample.

**5.1.5. Incubation:**

All petri dishes (except those for fecal coliform test) containing their respective membrane filters must be incubated within 30 minutes of the filtration step. The petri dishes are placed in separate plastic cakettes depending on the type of analysis, incubation period and incubation temperature. They are always incubated in an inverted position with the grid side of the membrane facing down. The bottom of the cakettes are lined with moistened paper towels to provide a humid atmosphere. Each cakette is labelled to indicate the type of analysis, the date and time of incubation and the date and time of counting colonies on the membranes.

**5.1.6. Counting:**

- 1) At the end of the incubation period, the bacterial colonies growing on the membranes are counted using a stereoscopic microscope with 10X magnification. Each petri dish is positioned on the slanted base and a cool white fluorescent light is set up to provide the best illumination.
- 2) Petri dishes should be observed and counts made within the designated incubation period. Prolonged incubation or standing of plates before observations are made could give inaccurate results, if colour differentiation is required for identification of a particular group of micro-organisms.

**5.2. Total Coliform Analysis:**

- 5.2.1. The m-Endo agar LES plates for the total coliform analysis are incubated at  $35 \pm 0.5^{\circ}\text{C}$  for  $22 \pm 2$  hours. The plates are counted immediately after removal from the incubator using the stereoscopic microscope at a magnification of 10X.
- 5.2.2. All colonies with a dull to bright, metallic, green-gold sheen are considered members of the coliform group. The sheen may consist of only a central spot or cover the entire colony. Colonies which lack sheen may be red, pink, blue, white or colourless and are considered as background colonies (non-coliforms). Doubtful colonies should be confirmed by transferring a small amount of inoculum from the colony to EC broth with incubation at  $35^{\circ}\text{C}$  for 24-48 hours. Gas production constitutes a positive result.
- 5.2.3. An ideal density range of colonies on a membrane filter would consist of a total count not exceeding 300 colonies and a coliform count of 20-80 colonies. Plates with conditions unsuitable for counting would be: those with sheen colony counts greater than 150; plates showing

confluent growth of colonies; control membranes with greater than 10 background colonies and/or one or more sheen colonies. Under these conditions, a repeat analysis should be made at a more appropriate dilution if the sample does not exceed the age limit or another sample should be requested.

- 5.2.4. If the total count exceeds 300 colonies/plate, but a suitable coliform count is obtained, background counts may be estimated by counting colonies on 10 squares of the membrane filter and multiplying the count by a factor of 10 as the membrane filter area is considered equivalent to 100 squares.

### 5.3. Fecal Coliform Analysis:

- 5.3.1. The m-TEC agar plates for fecal coliform analysis are incubated at  $44.5 \pm 0.5^{\circ}\text{C}$  for  $23 \pm 1$  hour. A gradual elevation of the m-TEC plates to the final incubation temperature of  $44.5^{\circ}\text{C}$  is achieved by incubating eight of the 100 mm square petri dishes in a plastic cakette along with two Phalar jars containing ice. The ice is prepared by dispensing 50 ml of tap water into each jar, and maintaining them overnight at  $-4^{\circ}\text{C}$ . The two ice jars should be placed at each end of the cakette, with the petri dishes (2 layers high) placed towards the centre of the cakette. Incubation of the cakettes should begin at least 2 hours after the first sample filtration and additional plates should not be added once the  $44.5^{\circ}\text{C}$  incubation has started. If insufficient filtrations were done to make up the eight petri dishes per cakette, blank agar plates should be added to obtain this number. Label each cakette properly to indicate the time of incubation and the time when counting should be started. Prolonged incubation may affect the appearance of the colonies. Plates should be counted immediately after removing from the incubation chamber.
- 5.3.2. Using the stereoscopic microscope at 10X, all yellow, yellow-brown, and yellow-green colonies are counted as fecal coliform colonies. The yellow to brown colour may consist of only a central spot for some colonies, or for other colonies the colour may cover the entire colony. Those colonies which are blue or gray or colourless should not be counted as fecal coliforms. Doubtful colonies should be confirmed in an EC broth medium, incubated at  $45.5^{\circ}\text{C}$  for 24 hours for evidence of gas production.
- 5.3.3. An ideal density range for fecal coliform counts would be 20-60 colonies per plate. Plates with greater than 150 fecal coliform colonies or confluent growth represent unsuitable plates for counting and the analysis should be repeated at a more appropriate dilution.

### 5.4. Fecal Streptococcus Analysis:

- 5.4.1. The m-Enterococcus agar plates for fecal streptococcus analysis are incubated at  $35 \pm 0.5^{\circ}\text{C}$  for  $48 \pm 3$  hours.
- 5.4.2. Using the stereoscopic microscope at 10X, all colonies that are red, maroon or pink are counted as fecal streptococci. Colourless, white or yellow colonies are not counted. Doubtful colonies should be confirmed in an Ethyl Violet Azide (EVA) medium incubated at  $35^{\circ}\text{C}$

for 48 hours. Turbid growth in this medium constitutes a positive result.

- 5.4.3. A suitable density range for fecal streptococci counts would be with plates having 20-100 colonies. Plates with greater than 150 fecal streptococci colonies or confluent growth are considered unsuitable and a higher dilution should be chosen for a repeat analysis, if the sample is not too old.

## **6. Calculation and Reporting:**

The coliform, background, fecal coliform and fecal streptococcus densities of a sample are recorded in terms of numbers of organisms per 100 ml. The general equation for calculating results would be:

$$\begin{array}{lcl} \text{Number of organisms} & = & \frac{100 \times \text{colonies counted}}{\text{per 100 ml} \qquad \qquad \text{ml sample filtered}} \end{array}$$

This calculation would be repeated for each type of indicator organism for which a count was obtained.

## **7. Preparation of Media for Microbiological Analysis:**

### **7.1. Medium for Total Coliform and Background Count Analysis**

The medium for conducting the total coliform and background count analysis is prepared from a dehydrated powder known as Bacto-m Endo agar LES (Difco). This medium is not autoclaved, therefore, aseptic conditions must be maintained throughout the preparation. Approximate preparation time is one hour.

#### **7.1.1. Apparatus for Preparation of Medium for Total Coliform and Background Count Analysis**

- 7.1.1.1. Thermometer, (0-110°C)
- 7.1.1.2. Stirring hot plates
- 7.1.1.3. Asbestos gloves
- 7.1.1.4. Balance, top loading, sensitive to 0.01 g
- 7.1.1.5. Bunsen burner
- 7.1.1.6. Pipettes, 10 ml, sterile
- 7.1.1.7. Spatula
- 7.1.1.8. Large stirring magnet
- 7.1.1.9. Graduated cylinder with aluminum foil cover, sterile, 1 liter
- 7.1.1.10. Large pair of forceps
- 7.1.1.11. Erlenmeyer flask, sterile, 2 liter with aluminum foil cover
- 7.1.1.12. Petri dishes, sterile, square plastic 100 x 15 mm (approx. 50-60)
- 7.1.1.13. Erlenmeyer flask, sterile, 1 liter with screw cap

- 7.1.1.14. One small fan
- 7.1.1.15. Beaker, 100 ml half filled with ethyl alcohol
- 7.1.2. **Reagents for Preparation of Total Coliform and Background Count Medium**
  - 7.1.2.1. Dehydrated m-Endo LES agar (Difco)
  - 7.1.2.2. Ethyl alcohol, 95%
  - 7.1.2.3. Distilled water in an Erlenmeyer flask, 1000 ml, sterile
- 7.1.3. **Preparation of 1 liter of m-Endo Agar LES Medium:**
  - 7.1.3.1. Check the balance to ensure that it is level and operational, zero the balance.
  - 7.1.3.2. Place the 2 liter Erlenmeyer flask on the balance and tare the balance to zero.
  - 7.1.3.3. Sterilize the clean spatula by dipping it in a 100 ml beaker half-filled with 95% ethyl alcohol and pass the spatula through the bunsen burner flame to burn off the alcohol.
  - 7.1.3.4. Weigh out 51 g of the dehydrated m-Endo agar LES powder into the 2 liter flask.
  - 7.1.3.5. Cover the mouth of the flask with aluminum foil.
  - 7.1.3.6. Flame the mouth of the 1 liter flask containing distilled water and the 1 liter graduate cylinder and measure out 1 liter of distilled water into the screw cap 1 liter flask.
  - 7.1.3.7. Using a 10 ml pipette, add 20 ml of 95% ethyl alcohol to the screw cap flask containing the measured 1 liter of sterile distilled water and gently mix the contents.
  - 7.1.3.8. Pour about 500 ml of the alcohol-water mixture into the flask containing the powdered medium and swirl the flask to thoroughly wet the medium, ensuring that none of the powdered medium is adhering to the bottom or sides of the flask.
  - 7.1.3.9. When the powdered medium is thoroughly wetted and mixed, the remainder of the alcohol-water mixture is added to the flask.
  - 7.1.3.10. Using the forceps, the stirring magnet is removed from a beaker of alcohol; flamed; and dropped into the flask.
  - 7.1.3.11. The flask is then placed on the stirring hot plate; the stirrer is activated to stir at medium speed; and the heat is turned on full.
  - 7.1.3.12. The thermometer is swabbed with alcohol and the temperature of the flask is checked as the temperature rises until a temperature of 94-95°C is reached.
  - 7.1.3.13. By the time, the above temperature has been reached, the medium should be totally in solution and be a dark red wine colour. The flask is removed from the hot plate and transferred to another hot plate which just has the stirring

device activated. The fan should be moved into position to assist with the cooling of the medium to about 45-50°C or until the flask can be grasped comfortably in one's hands.

- 7.1.3.14. During the heating process, the 100 x 15 mm petri dishes are laid out along the bench, previously swabbed with dettol. When the medium is cooled to 45-50°C, about 15-20 ml of the medium is poured aseptically into each petri dish and the lid of each dish is left slightly ajar to facilitate solidification of the medium without excess condensation on the lid of each petri dish.
- 7.1.3.15. When the medium has solidified, the lids are closed; the petri dishes are inverted and placed in covered cakettes in a refrigerator at 4°C until required for analysis. The date when the medium was made is marked on each cakette. The plates should be kept in total darkness in the refrigerator at 4-5°C and stored for no longer than 4 days before being used.
- 7.1.3.16. Clean up the preparation area, making sure that the hot plate, stirring mechanisms, and the weighing scale have also been cleaned up and turned off.

## **7.2. Medium for Fecal Coliform Analysis**

The medium for conducting fecal coliform analyses is prepared from individual ingredients according to a formulation by Dufour, Strickland, and Cabelli (8.4.) and is known as m-TEC medium.

### **7.2.1. Apparatus for Preparation of Fecal Coliform Medium:**

- 7.2.1.1. Glass beaker, 2 liter
- 7.2.1.2. Graduated cylinder, 1 liter
- 7.2.1.3. Weighing spatula, large and small
- 7.2.1.4. Large stirring magnet
- 7.2.1.5. Stirring hot plates
- 7.2.1.6. Thermometer (0-110°C)
- 7.2.1.7. Asbestos gloves
- 7.2.1.8. Balance, top loading, sensitive to 0.01 g
- 7.2.1.9. Analytical balance, sensitive, to 0.0001 g
- 7.2.1.10. Weighing boats and weighing papers
- 7.2.1.11. Bunsen burner
- 7.2.1.12. Large pair of forceps
- 7.2.1.13. Beaker, 600 ml, sterile
- 7.2.1.14. Petri dishes, sterile, plastic, 100 x 15 mm square (approx. 50)
- 7.2.1.15. Fan
- 7.2.1.16. Aluminum foil

7.2.1.17. Plastic cakette

7.2.1.18. pH meter

**7.2.2. Reagents for Preparation of Fecal Coliform Medium**

7.2.2.1. Proteose peptone No. 3 (5.0 g/l)

7.2.2.2. Yeast extract (3.0 g/l)

7.2.2.3. Lactose (10.0 g/l)

7.2.2.4. Sodium chloride (NaCl), reagent grade crystals (7.5 g/l)

7.2.2.5. Dipotassium hydrogen phosphate ( $K_2HPO_4$ ), reagent grade (3.3 g/l)

7.2.2.6. Potassium dihydrogen phosphate ( $KH_2PO_4$ ), reagent grade powder (1.0 g/l)

7.2.2.7. Sodium lauryl sulphate ( $C_{12}H_{25}OSO_3Na$ ) reagent grade (0.2 g/l)

7.2.2.8. Sodium desoxycholate ( $C_{24}H_{39}ONa$ ) (0.1 g/l)

7.2.2.9. Bromocresol purple (0.08 g/l)

7.2.2.10. Bromophenol red (0.08 g/l)

7.2.2.11. Agar (15.0 g/l)

7.2.2.12. Distilled water (1000 ml)

7.2.2.13. Sodium hydroxide solution (1 N), see 4.4.

**7.2.3. Preparation of 1 Liter of m-TEC Medium**

7.2.3.1. Check the balance to ensure that it is level and operational, zero the balance.

7.2.3.2. Using the weighing boats weigh out the ingredients 7.2.2.1. to 7.2.2.6. using the top load balance.

7.2.3.3. Measure 1 liter of distilled water into the 2 l beaker and place it on the stirring device. Add a clean stirring magnet and activate the stirrer to run at medium speed.

7.2.3.4. Add each of the ingredients separately and wait until each has dissolved before adding another.

7.2.3.5. Using the weighing papers weigh out the ingredients 7.2.2.7. to 7.2.2.10. on the analytical balance and add to the medium.

7.2.3.6. Weigh out the agar on the top loading balance and add to the medium.

7.2.3.7. Adjust pH to 7.2 with 1N NaOH prior to heating.

7.2.3.8. Place the 2 l beaker on a stirring hot plate and heat to 90°C to dissolve the agar and other ingredients. The 2 l beaker is covered with aluminum foil and a thermometer is positioned through the aluminum foil during the heating process to monitor the temperature.

7.2.3.9. After the medium has reached 90°C, the thermometer is removed and the opening is sealed with autoclave tape. The medium is then autoclaved for 15 minutes at 121°C.



- 7.2.3.10 Following sterilization, the 2 l beaker is placed on a stirring device in front of a fan and allowed to cool to 60 °C.
- 7.2.3.11. Pour about 500 ml of the sterile medium into the sterile 600 ml beaker for convenience in pouring the medium into the 100 mm square petri dishes. Flame thoroughly around the aluminum foil and lips of each beaker before and after the transfer of the medium. Place the remainder in the 2 l beaker back on the stirring device until needed.
- 7.2.3.12. Using the smaller 600 ml beaker, pour about 20-25 ml of medium into each petri dish to give a medium depth of 3-4 mm. Leave the lids of the petri dishes about one quarter open to allow cooling and solidification of the medium without excess condensation forming on the lids. Continue to pour the plates until all the medium is dispensed.
- 7.2.3.13. After about 20 minutes, the medium should be thoroughly solidified and the petri dish lids should be closed; the petri dishes should then be stacked in an inverted position in cakettes; the plates are stored in a darkened area at room temperature for 24 hours to check for contamination before placing them in the refrigerator at 4 °C.
- 7.2.3.14. Each cakette is labelled with the date of preparation and the plates may be stored at refrigeration temperature in the moisture tight containers for up to 4 weeks.
- 7.2.3.15. Clean up the preparation area including the weighing scales, stirring devices and work bench. Wash the beakers in hot water to remove all traces of the agar medium.

### 7.3. Medium for Fecal Streptococcus Analysis

The medium for conducting fecal streptococcus analysis is prepared from a dehydrated powder form of m-Enterococcus agar (Difco). This medium is not autoclaved, therefore, aseptic conditions must be maintained throughout the preparation. Approximate preparation time is one hour.

#### 7.3.1. Apparatus for Fecal Streptococcus Medium

- 7.3.1.1. Erlenmeyer flask, sterile, wide mouth, 1 liter with aluminum foil cover
- 7.3.1.2. Balance, top loading, accurate to 0.01 g
- 7.3.1.3. Bunsen burner
- 7.3.1.4. Stirring hot plates
- 7.3.1.5. Weighing boats
- 7.3.1.6. Thermometer, (0-110°C)
- 7.3.1.7. Asbestos gloves
- 7.3.1.8. Large stirring magnet
- 7.3.1.9. Graduated cylinder, sterile, 500 ml
- 7.3.1.10. Spatula
- 7.3.1.11. Large pair of forceps



- 7.3.1.12. Petri dishes, sterile square plastic 100 mm size (approx. 25)
- 7.3.1.13. Small fan
- 7.3.1.14. Beaker, 100 ml, half filled with ethyl alcohol
- 7.3.2. **Reagents for Preparation of the Fecal Steptococcus Medium**
  - 7.3.2.1. Dehydrated m-Enterococcus agar (Difco)
  - 7.3.2.2. Ethyl alcohol, 95%
  - 7.3.2.3. Sterile distilled water in 1 liter flask
- 7.3.3. **Preparation of 500 ml of m-Enterococcus Agar Medium:**
  - 7.3.3.1. Check the balance to ensure that it is level and operational, zero the balance.
  - 7.3.3.2. Place the weighing boat on the balance and tare the balance to zero.
  - 7.3.3.3. Sterilize the spatula by dipping it in alcohol and passing it through the bunsen burner flame.
  - 7.3.3.4. Weigh out 21 g of dehydrated m-Enterococcus agar powder into the weighing boat.
  - 7.3.3.5. Using aseptic procedure, remove the aluminum foil cover and flame the mouth of the 1 liter Erlenmeyer flask. Carefully transfer the powder in the weighing boat to this flask and recover it.
  - 7.3.3.6. Remove the cap from the sterile distilled water flask, flame the mouth and measure 500 ml into the sterile graduate cylinder.
  - 7.3.3.7. Aseptically add the 500 ml of sterile distilled water to the flask containing the powdered medium and gently swirl the flask to ensure that none of the powder medium is adhering to the bottom or sides of the flasks.
  - 7.3.3.8. Alcohol flame the large stirring magnet and place it in the flask.
  - 7.3.3.9. The flask is then placed on the stirring hot plate; the stirrer is activated to stir at medium speed; and the heat is turned on full.
  - 7.3.3.10. The thermometer is swabbed with alcohol and the temperature of the flask is checked as the temperature rises until a temperature of 91-92°C is reached.
  - 7.3.3.11. By the time, the flask has reached the above temperature, the medium should be totally in solution and be a clear, straw colour. The flask is removed from the hot plate and transferred to the other hot plate which just has the stirring device activated. The fan should be moved into position to assist with rapid cooling of the medium to about 55°C.
  - 7.3.3.12. During the heating process, the 100 mm square petri dishes are laid out along the bench previously swabbed with dettol.

- 7.3.3.13. The flask containing the cooled, but still warm, medium is removed from the stirrer and the lid of each petri dish is opened sufficiently to pour in about 20 ml of the liquid medium. The lid of the petri dish is left about one quarter open to minimize condensation during the cooling and solidification process.
- 7.3.3.14. The petri dishes are left standing until the medium is solidified, after which the lids of the petri dishes are closed and they are inverted and packed in a cakette, which is labelled with the name of the medium and preparation date. The petri dishes are stored at room temperature until quality control tests have been done after which they are placed in the refrigerator at 4°C for a period not exceeding one month.
- 7.3.3.15. Clean up the preparation area, making sure that the hot plates, stirring mechanisms, and the weighing scale have been cleaned up and turned off.
- 7.3.3.16. Every effort should be made to avoid overheating of this medium during its preparation.

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